UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

HOMEOSTASIS OF THE T CELL MEMORY COMPARTMENT

Catarina de Carvalho Soares Dinis Leitão

Tese orientada por :
Professor Doutor António A. Freitas
e Professora Doutora Maria Gabriela Rodrigues

DOUTORAMENTO EM BIOLOGIA
(Biologia Celular)

2008

FINANCIAL SUPPORT:

O trabalho apresentado nesta tese foi realizado com o poio financeiro da Fundação para a Ciência e a Técnologia (bolsa de referência SFRH/BD/16762/2004)

AKNOWLEDGEMENTS

Esta tese é dedicada especialmente aos meus pais como agradecimento por sempre me terem apoiado nesta aventura e por sempre terem tido uma palavra lúcida e crítica em momentos fundamentais. Também a dedico ao meu avô António que sempre me ensinou que o conhecimento nunca é demais e que devemos estar sempre ávidos em aprender novas coisas.

I would like to thank to Professor António Freitas for giving me the opportunity to make the PhD in his prestigious laboratory and in the prestigious Pasteur Institute, and for having contributed always with good suggestions for the project.

I would like to thank my supervisor Sylvie Garcia for all her support during the thesis, for the brainstorm discussions, for the non-stopping ideas and suggestions for the project and for teaching me to look at the negative results or at the problems that occurred during the thesis in a positive way.

I would like to thank my Professor Maria Gabriela Rodrigues for having accepted to be my supervisor in Portugal and to be the link between Pasteur Institute and F.C.U.L.; and for having helped me so immediately each time I had doubts or problems to resolve.

I would like to thank Marie-Christine Vougny for being such an efficient secretary that allowed me to not get "lost" in Paris.

I would like to thank Christian Vosshenrich for having teached me to use FlowJo software and for being always my "angel guard" when FACS machines were broken.

I would like to thank Gernot Sellge, in concerning to bacteria preparation and for the initial discussions and suggestions about the Attrition project.

I would like to thank all the rest of the members of my laboratory in general, but particularly four that have accompanied me, basically, during all my thesis and that have proportioned a great ambience in the laboratory: Bruno, Caroline, Malika and Yi.

I would also like to thank all my friends (including family), the ones from Portugal and the new ones that I made here, for all their support over these four years of thesis. I'm not going to mention names, because it is an endless list, but they know I'm grateful.

TABLE OF CONTENTS

RESUMO **6**

ABSTRACT **11**

ABBREVIATIONS **12**

INTRODUCTION **14**

1 THYMIC DEVELOPMENT OF T CELLS **16**

1.1 THE ROLE OF SP-MHC 17
1.2 THE ROLE OF CYTOKINES 18

2 PERIPHERAL NAIVE T CELL POOL **19**

2.1 NAIVE T CELL CHARACTERIZATION 19
2.2 HOMEOSTASIS OF NAIVE T CELLS 19
2.2.1 The role of p-MHC *20*
2.2.2 The role of cytokines *22*

3 PERIPHERAL ACTIVATED/MEMORY T CELL POOL **23**

3.1 ACTIVATED/MEMORY T CELL CHARACTERIZATION 23
3.2 ACTIVATED/MEMORY T CELL HOMEOSTASIS 24
3.2.1 Generation of activated/memory T cells in the classical activation situation (infection) *25*
a) Initial activation 26
b) T-cell expansion and differentiation 27
c) Contraction 28
d) Memory phase 30
3.2.2 Generation of activated/memory T cells in lymphopenic environments (LDP) *38*
a) Initial activation 39
b) T cell proliferation/ differentiation 41
c) memory-like phase 42
3.2.3 Survival/maintenance of memory T cells *43*
a) The role of p-MHC 43
b) The role of cytokines 45
3.2.4 Activation of memory T cells *46*
a) Recall response to infections (conventional activation of memory T cells) 46
b) LDP of memory T cells 47
3.3 T CELL ATTRITION 49
3.3.1 Attrition models *51*
3.3.2 Attrition mechanism *51*

4 SALMONELLA THYPHIMURIUM **53**

IN THIS THESIS: **56**

SUBMITTED ARTICLE **57**

ADDITIONAL RESULTS **81**

1 MEMORY T CELL ATTRITION **82**

1.1 IN THE ABSENCE OF INTENTIONAL IMMUNIZATION : « NATURAL ATTRITION » 82
1.2 DURING BACTERIAL INFECTION: “SALMONELLA-INDUCED ATTRITION”: 88

2 MATERIALS AND METHODS **97**

DISCUSSION **100**

1 OVERLAP BETWEEN SURVIVAL AND LDP MECHANISMS: COMPETITION FOR P-MHC **101**

2 INTERDEPENDENCE BETWEEN NAIVE AND MEMORY T CELL COMPARTMENTS. **106**

3 IMPORTANCE OF TCR DEGENERACY IN PERIPHERAL HOMEOSTASIS AND CONSEQUENCE FOR THE T CELL POOL. 108

4 ANOTHER CASE: MEMORY ATTRITION INDUCED BY INFECTION 109

5 ATTRITION: CELL MIGRATION INTO OTHER ORGANS VERSUS APOPTOSIS 111

6 POSSIBLE MECHANISMS OF ATTRITION 112

7 FUTURE PERSPECTIVES 114

BIBLIOGRAPHY 116

RESUMO

O número de linfócitos T é mantido constante no organismo, apesar de haver uma produção diária e de haver uma proliferação de células T específicas após contacto com o seu antigénio nominal. Este equilíbrio é designado homeostasia. Subjacente ao controlo homeostático do número de células T encontra-se o conceito de competição por recursos limitantes. Cada nova célula T (produzida no timo) tem de competir com outra célula nova e/ou células T residentes na periferia para sobreviver. A periferia é um termo geral que engloba todos os locais onde as células T residem após abandonarem o timo.

As células T αβ podem ser subdivididas em duas populações com base na expressão do seu co-receptor. Deste modo existem as subpopulações dos linfócitos T $CD4^+$ e $CD8^+$. Estas duas populações, por sua vez, dependendo do grau de diferenciação que apresentam na periferia, podem formar dois compartimentos celulares distintos. Um compartimento celular é designado "naïve" pelo facto de representar o conjunto de linfócitos T que nunca estiveram em contacto com os seus antigénios específicos. O outro é denominado compartimento "activado/memória", por conter células que interargiram com antigénios específicos responsáveis pela sua activação e diferenciação em células efectoras e/ou células memória. Pensa-se que estes compartimentos têm uma regulação homeostática independente, embora existam evidências que eles partilhem, em parte, alguns recursos comuns.

Esta tese tem como objectivo o estudo da homeostasia das células T, com um interesse particular na população das células activada/memória, tanto em estado de equílibrio, como quando uma perturbação a este equilíbrio é introduzida (infecção bacteriana).

Na primeira parte do trabalho decidimos investigar se as células T que apresentam um fenótipo naive e as células T que apresentam um fenótipo activado ou memória, e que, portanto pertencem a compartimentos celulares diferentes, podem competir entre si por recursos comuns. Usando diferentes abordagens mostrámos que, na realidade, o reconhecimento de recursos específicos ao receptor das células T (TCR), muito provavelmente o complexo péptido-complexo maior de histocompatibilidade (p-MHC), sobrepõe-se entre diferentes populações de células T pertencentes a diferentes compartimentos e submetidas a mecanismos homeostáticos distintos.

A transferência de linfócitos T em hospedeiros linfopénicos, isto é, hospedeiros contento poucas ou nenhumas células T, induz a proliferação das células dadoras. Este fenómeno é

designado proliferação induzida pela linfopénia (em inglês lymphopenia driven proliferation-LDP). Contudo, verificámos que nem todas as células T $CD8^+$ policlonais transferidas em ratinhos $RAG2^{-/-}$ (recombination activation gene 2) e transgénicos (Tg) para diferentes TCRs restrictos aos antigénios do MHC classe I, são capazes de proliferar, apesar de sobreviverem. Observámos adicionalmente que o grau de inibição de LDP está correlacionado positivamente com o número absoluto de células T residentes.

A ausência de proliferação de uma fracção de células dadoras tanto poderia ser devido a uma competição por recursos específicos ao TCR (por exemplo p-MHC) ou por recursos inespecíficos (por exemplo citocinas). Concluímos que as células dadoras e as residentes competem para interargir com os mesmos p-MHC, pelo facto de se ter verificado que a fracção de células T policlonais que não foram capazes de expandir num determinado ratinho $RAG^{-/-}$ com TCR Tg, expandem quando transferidas para ratinhos $RAG^{-/-}$ com outro TCR Tg ou para ratinhos $RAG^{-/-}$. Esta observação, no entanto, não elimina a possibilidade de haver igualmente uma competição por recursos não TCR específicos, como citocinas. Contudo, o facto de se ter observado que a fracção de células que não se dividiam num hospedeiro Tg para um determinado TCR, são capazes de proliferar e de atingir valores semelhantes tanto em ratinhos com um TCR tg diferente do do primeiro hospedeiro, como em ratinhos sem células T, sugere que as citocinas como IL-7, entre outras, não se encontram em condições limitantes. A competição por p-MHC, entre algumas células policlonais e as células naive do ratinho hospedeiro, aplica-se tanto às células T dadoras com fenótipo naive como com fenótipo activado/memória, questionando assim a regulação homeostática independente entre os dois compartimentos celulares periféricos. Visto que certas células T $CD8^+$ policlonais não podem proliferar devido à presença das células T $CD8^+$ monoclonais do hospedeiro, questionámo-nos sobre o que aconteceria se co-injectássemos um igual número de células não capazes de proliferar num determinado recipiente com as células desse recipiente num ratinho $RAG^{-/-}$. Nesta situação, em que ambos os tipos de células estão submetidos simultaneamente a uma mesma condição, as células policlonais proliferam e atingem valores absolutos finais semelhantes tanto quando são co-injectadas, como quando injectadas sozinhas. Pelo contrário, o número de células T $CD8^+$ monoclonais recuperado no grupo onde ocorreu a co-injecção é inferior ao obtido no grupo controlo. Assim, podemos concluir que nesta situação, as células T $CD8^+$ policlonais excluem as células T $CD8^+$ monoclonais, ao competirem por "interargir" com os mesmos p-MHC.

Posteriormente, decidimos estudar o papel dos recursos p-MHC no destino de novas células T $CD8^+$ maturas introduzidas num hospedeiro que contém células T $CD8^+$ residentes que resultam de LDP. Para este fim, usámos transferências adoptivas sequenciais de células T com TCR Tg em hospedeiros com TCR Tg homólogo ou heterólogo. Quatro semanas após a segunda transferência, observámos que a presença da primeira população inibe a expansão da segunda, sendo esta inibição maior quando a segunda população apresenta o mesmo TCR que as células T residentes. A comparação dos perfis de CFSE (Carboxyfluorescein succinimidyl ester) confirmou um atraso na proliferação das células T transferidas quando as células residentes apresentam a mesma especificidade. Estes resultados indicam que, apesar de ambas as populações de células T competirem por p-MHC, outros recursos, possivelmente citocinas, são necessários, pelo menos, para a acumulação final da segunda população de células T derivadas de LDP.

A importância do p-MHC foi também estudada quando a nova população introduzida de células T derivou da medula óssea. As células T residentes continuaram a ser derivadas de LDP. Como protocolo, primeiramente transferimos células T maturas com um TCR Tg num ratinho $RAG^{-/-}$, e quatro semanas depois transferimos progenitores de células T da medula óssea, de ratinhos dadores com TCR Tg, com a mesma ou com diferente especificidade em relação à primeira população. Em contraste com a situação anterior, onde assistimos a uma inibição da acumulação da segunda população independentemente da especificidade do clone de células T residentes derivadas de LDP; neste caso o decréscimo do número de células T da segunda população injectada, ocorre apenas significativamente quando as células residentes (derivadas de LDP) têm um TCR com a mesma especificidade. Assim, podemos concluir que as células T derivadas de LDP e as células T derivadas da medula óssea competem por p-MHC.

De referir que, qualquer que seja a natureza da nova população introduzida, tanto células T maturas como células T derivadas da medula óssea, a população de células T residentes derivadas de LDP não é afectada pelo aparecimento da nova população.

Na segunda parte deste estudo investigámos as condições nas quais células T memória pre-existentes pudessem ser afectadas, tanto na ausência como na presença de imunização intencional. Na ausência de imunização, observámos que as células T memória podem ser substituídas por células T $CD8^+$ ou $CD4^+$ derivadas da medula óssea, no entanto este facto só ocorre quando a degenerescência do TCR destas células é suficientemente elevada. Estes resultados indicam, mais uma vez, que as células T naive e activada/memória não são

completamente independentes. Contudo, nesta situação não são conhecidos os recursos pelos quais estes dois tipos de células competem. Visto que há uma redução do número de células T memória, referimo-nos a este fenómeno como "atrição natural", em oposição à atrição previamente descrita (Selin et al., 1999) e que consiste no empobrecimento do compartimento de células memória T pre-existentes após infecções virais. Esta atrição foi sempre observada no compartimento das células T memória e para o subconjunto das células $CD8^+$, tanto em infecções virais agudas como em infecções com a *Listeria monocytogenes* (LM). Sabendo que, tanto em infecções virais como em infecções com LM, as células T $CD8^+$ expandem mais vigorosamente que as células T $CD4^+$, é possível que haja uma maior pressão selectiva sobre as células T $CD8^+$ que sobre as células T $CD4^+$, a nível da atrição. Com o objectivo de estudar se a atrição pode também afectar células T $CD4^+$ em caso de infecção, decidimos usar como agente patogénico, Salmonella enterica serovar Typhimurium (daqui em diante referida como Salmonella), que, ao contrário da maioria das infecções, induz uma proliferação massiva de células T $CD4^+$ (Srinivasan et al., 2004) e uma expansão e contracção retardada das células T $CD8^+$ (Luu et al., 2006). A resolução da infecção originada pela Salmonella, em oposição às infecções virais ou com a LM, é criticamente dependente da activacção das células T $CD4^+$ (Hess et al., 1996; Mastroeni et al., 1992; Nauciel, 1990; Pie et al., 1997; Sinha et al., 1997). Através da geração de ratinhos quiméricos que têm um sistema adaptativo constituído apenas por células T $CD8^+$ aHY e células T $CD4^+$ Marilyn memória (reconhecem epítopos do complexo de histocompatibilidade menor codificado pelo cromossoma Y), resolvemos analisar o destino dessas células T memória não específicas após infecção com Salmonella. Duas semanas após a infecção, o número de células T $CD4^+$ e $CD8^+$ diminuiu no baço. Esta diminuição está relacionada com a morte celular e não devido a uma simples redistribuição dessas células por outros órgãos. Observámos também que a atrição das células T está correlacionada com a quantidade de bactéria presente no órgão.

Pelo facto de ter sido sugerido que interferões (IFNs) do tipo I desempenham uma função na atrição das células T memória, decidimos determinar se estas moléculas têm um efeito directo sobre as células T memória aHY e Marilyn através da geração de ratinhos quiméricos contendo células T memória deficientes para o receptor de IFNs do tipo I ($IFNAR^{-/-}$). A infecção destes ratinhos quiméricos com Salmonella originou mais uma vez a redução do número de células T não específicas, indicando que IFNs do tipo I não desempenham um papel directo na morte celular.

Como conclusão, estes resultados permitiram-nos mostrar algumas das regras que possivelmente contribuem para modelar o estabelecimento do reportório das células T na periferia, no estado de equilíbrio, revelando que os recursos importantes para os diferentes compartimentos das células T sobrepõem-se entre si. No caso de infecção, o reportório e/ou a frequência de células T memória pode também ser afectado. Apesar de termos descrito algumas características da atrição com o modelo de infecção da Salmonella, ainda não podemos responder se há uma atrição selectiva no que diz respeito a numerosos parâmetros, nem definir os mecanismos responsáveis pela ocorrência deste fenómeno. Assim sendo, os estudos devem ser continuados para atingirmos a compreensão completa do fenómeno de atrição.

Palavras-chave: proliferação induzida pela linfopénia (LDP), atrição, competição, células T, péptido-complexo de histocompatibilidade maior (p-MHC)

ABSTRACT

In spite of daily T cell production in the thymus associated with intensive proliferation and differentiation of specific T cells upon each antigenic stimulation, peripheral T cells numbers are kept constant. This equilibrium is called homeostasis and subjacent to this process is the concept of competition for limiting resources. Each newly produced T cell has to compete with other new and/or resident peripheral T cell to survive. The periphery comprises two main compartments, the naive and activated/memory T cell pools, for each $CD8^+$ and $CD4^+$ subsets. These compartments are thought to have independent homeostatic regulation, although they share some common resources. The purpose of this thesis is to study the homeostasis of T cells, with a particular interest for the memory subsets, either at steady state or after disruption of this equilibrium upon infection.

In the first part of the work, we showed that T cells belonging to different peripheral compartments could compete with each other for p-MHC (peptide-MHC complexes), even if they present a distinct T cell receptor. Moreover, we observed that recognition of p-MHC overlaps not only between different T cell populations but also between T cells ongoing different homeostatic mechanisms such as survival, LDP or accumulation after thymic emigration.

In the second part of the study, we show again a modulation of T cell repertoire due to the displacement of memory T cells by BM-derived T cells presenting degenerate TCR. Besides this steady state attrition termed “natural attrition”, we proposed to study the fate of memory T cells upon Salmonella thymimurium infection. Preliminary data showed that both non-specific $CD4^+$ and $CD8^+$ memory T cells were depleted upon this infection and that type I IFN were not directly implicated in this death. More work remains to be performed to precisely define the targets and mechanism of this attrition.

Key words: lymphopenia driven proliferation (LDP), attrition, competition, T cells, peptide-major histocompatibility complex (p-MHC)

ABBREVIATIONS

Ag: antigen

AICD: activation induced cell death

APCs: antigen presenting cells

Bcl-2: B-cell lymphoma 2

Blimp-1: B lymphocyte-induced maturation protein-1

BM: bone marrow

BMDCs: bone marrow derived dendritic cells

cDNA: complementary DNA

CFSE: Carboxyfluorescein succinimidyl ester

CFU: colony forming units

CTLs: cytotoxic T lymphocytes

DCs: dendritic cells

DISC: death-inducing signaling complex

DN: double negative

DP: double positive

FDCs: Folicular dendritic cells

GFP: green fluorescent protein

IEX-1: immediate early response gene X-1

IFN: interferon

IFNAR: interferon α/β receptor knockout

IL: interleukin

LCMV: lymphocytic choriomeningitis virus

LCP: lineage committed progenitors

LDP: lymphopenia driven proliferation

LM: *Listeria monocytogenes*

LNs: lymph nodes

LPS: lipopolysaccaride

Lt-HSC: long-term hematopoietic stem cells

mAb: monoclonal antibody

MHC: major histocompatibility complex

NFκB: nuclear factor-kappa B

NOD: nucleotide-binding oligomerization domain

OVA: ovalbumin

PAMPs: pathogen associated molecular patterns

PCR: polymerase chain reaction

PFU: plate forming units

p-MHC: peptide-MHC

poly(I:C): polynosinic:polycytidylic acid

PRR: pathogen recognition receptors

RAG: recombinase activating gene

rIL-7: recombinant IL-7

RNA: Ribonucleic acid

ROS: reactive oxygen species

SP: single positive

Spi2A: Serine protease inhibitor 2A

sp-MHC: self peptide – MHC

St-HSC: short-term hematopoietic stem cells

T-bet: Tata-box expressed in T cells

T_{CM}: Central memory T cells

TCR: T cell receptor

T_{EM}: Effector memory T cells

Tg: transgenic

TGF-β: transforming growth factor-β

Th: T helper cells

TLR: toll like receptors

TNF: tumor necrosis factor

TNFR: tumor necrosis factor receptor

TRAIL: tumor-necrosis factor related apoptosis-inducing ligand

Wt: wild-type

VSV: vesicular stomatitis virus

INTRODUCTION

INTRODUCTION

In the 19th century it was realized for the first time that the body has control mechanisms able to maintain an internal equilibrium in spite of changes in the environment (Bernard, 1865). This type of control was later called homeostasis (Cannon, 1932). This concept also applies to the immune system and implies that the introduction of a perturbation may modify the number of immune cells, but once the perturbation is removed, the number of cells tends to return to the previous levels.

The size of the lymphocyte pool is of crucial importance to the adaptive immune system. On the one hand, this pool must be sufficiently diverse to detect and destroy a wide range of potential pathogens; on the other hand, there is only limited physical space in the body to house all of these cells. Therefore, any cell that is produced only survives if another dies: homeostasis introduces competition between lymphocytes for limited resources (Freitas and Rocha, 1993; Freitas and Rocha, 2000).

T cell homeostasis occurs from thymocyte production to peripheral compartmentalization.

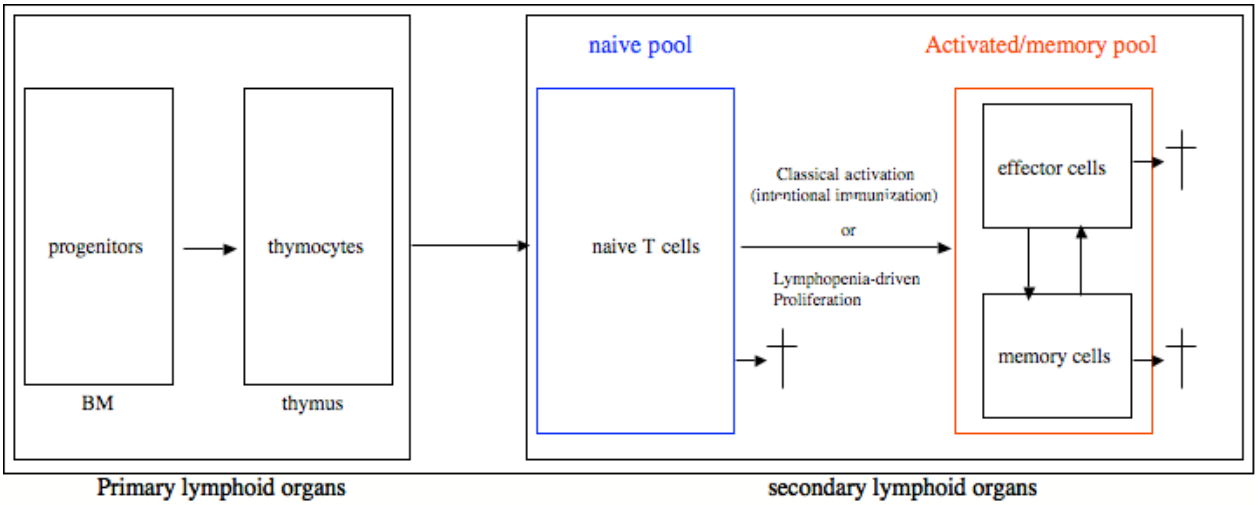


Figure 1: **T cell homeostasis**. Scheme that shows the different compartments of T cells from development in the primary lymphoid organs to final differentiation in the periphery, in an adult individual.

Bernard. C. (1865). Introduction à l'étude de la médecine expérimentale. J.-B Baillière

Cannon. WB. (1932) The wisdom of the body. Norton.

1 THYMIC DEVELOPMENT OF T CELLS

All the cellular elements of the blood, including red blood cells, platelets and white blood cells of the immune system, derive ultimately from the same precursors named hematopoietic stem cells (HSC) that are present in the liver of the fetus and in the bone marrow (BM) of newborns as well as adults. T cells develop from a common lymphoid progenitor in the bone marrow that also gives rise to B cells, but those progeny destined to become T cells leave the bone marrow and migrate to the thymus (Donskoy and Goldschneider, 1992).

Two lineages of T cells (αβ and γδ) are generated in the thymus, defined by the expression of distinct αβ or γδ T cell receptor (TCR) complexes (Petrie et al., 1992). The development of αβ T cells in the thymus is characterized by the ordered expression of several surface molecules (including the co-receptors CD4 and CD8) and proceeds through well-defined stages of proliferation, differentiation and regulated cell death before giving rise to mature αβ T cells. There are three main stages of development: an early double negative (DN; $CD4^-CD8^-$) stage, a predominant double positive (DP: $CD4^+CD8^+$) stage and mature single positive (SP) $CD4^+$ or $CD8^+$ cell stage. The immature non-TCR-bearing DN subset is classically subdivided into four successive developmental stages on the basis of CD44, CD117 (c-kit) and CD25 expression (Godfrey et al., 1993; Zuniga-Pflucker and Lenardo, 1996). TCRβ rearrangement is initially detected in DN2 cells and continues predominantly during the mostly non-cycling DN3 stage and is mediated by the enzymatic activity of the proteins encoded by recombination-activating gene (RAG) 1 and RAG2 genes (Godfrey et al., 1994; Capone et al., 1998). Mice lacking either of these genes cannot form receptors and thus have no lymphocytes.

Immature DN thymocytes upregulate the co-receptors upon successful assembly of the complex composed of CD3, TCRβ chain and pre-TCRα chain. At the DP stage, TCRα chain rearrangement is initiated and thymocytes become eligible for both positive and negative selection (Sebzda et al., 1999). Positive and negative selections occur according to the strength of signal that thymocytes receive through their TCR. In general, it appears that developing lymphocytes whose receptors interact weakly with self-antigens receive signals that enable them to survive; this type of selection is known as positive selection (von Boehmer, 1994). In contrast, lymphocytes whose receptors bind strongly to self-antigens receive signals that lead to their

death; this is termed negative selection (Nossal, 1994). The lymphocytes that survive to form the mature SP lymphocyte population are thus only a small fraction of those generated in the bone marrow and thymus.

1.1 The role of sp-MHC

Numerous studies have shown that positive and negative selection involves combinatorial recognition of self-peptides (sp) bound to the MHC (Nikolic-Zugic and Bevan, 1990; Jameson et al., 1995).

Positive selection was discovered with the generation of aHY TCR transgenic mice (von Boehmer, 1990). Analysis of developing thymocytes expressing defined TCRs clearly showed that MHC class I restricted thymocytes were skewed toward the $CD8^+$ SP lineage (Sha et al., 1988; Teh et al., 1988), whereas class II-restricted TCR transgenic thymocytes were selected to the $CD4^+$ lineage (Berg et al., 1989; Kaye et al., 1989). The notion that positive selection was required for T cell development was reinforced by data from various gene knockout mice showing that mice impaired for the class I MHC expression fail to develop mature $CD8^+$ T cells (Koller et al., 1990; Zijlstra et al., 1990; Van Kaer et al., 1992), whereas mice lacking class II expression have a selective deficiency in $CD4^+$ T cell development (Cosgrove et al., 1991; Grusby et al., 1991).

Two models have been proposed for the T cell interaction during selection:

-The qualitative/peptide model proposes that mutually exclusive qualitatively different peptides promote positive and negative selection (Mannie, 1991; Janeway, 1992; Jameson et al., 1995). This model was primarily supported by studies demonstrating that nonstimulatory antagonist peptides could promote positive selection (De Magistris et al., 1992; Hogquist et al., 1994; Jameson et al., 1994), while agonist peptides were shown to promote clonal deletion. However, several experiments have questioned these initial observations (Page et al., 1994; Spain et al., 1994; Ignatowicz et al., 1996).

-The avidity model proposes that positive selection is the result of low avidity thymocyte interactions, whereas high avidity interactions elicit negative selection (Ashton-Rickardt and

Tonegawa, 1994; Williams et al., 1997). Cells bearing TCRs with no affinity for sp-MHC molecules die by neglect. The key parameters that govern cell fate are TCR affinity for sp-MHC and the overall avidity of the TCR, such as the expression level of co-receptors and adhesion molecules, for these ligands. In this model, multiple TCR/sp-MHC interactions are integrated to form a signaling gradient that defines cell fate. Numerous weak TCR-ligand binding events or limited high affinity interactions provide sufficient avidity to induce an integrated signaling cascade that reaches a positive selection threshold. Direct evidence for this model were the studies indicating that different concentrations of the same peptide can mediate both positive and negative selection (Sebzda et al., 1994; Cook et al., 1997; Fukui et al., 1997).

1.2 The role of cytokines

Much attention has been devoted to TCR/sp-MHC interactions, which mediate positive and negative thymic selection at the level of DP immature T cells. However, prior to this stage, T lineage precursors undertake an extremely complex course of proliferation and differentiation (Godfrey and Zlotnik, 1993).

Examination of anti-IL7 mAb-treated mice (Grabstein et al., 1993), as well as IL-7R$\alpha^{-/-}$ (Peschon et al., 1994) and IL-7$^{-/-}$ (von Freeden-Jeffry et al., 1995) mice that exhibit an early defect in lymphopoiesis, has revealed an important role of this cytokine in T cell development.
IL-7 belongs to the common γ chain (γc) cytokine family, along with (IL) -2, -3, -4, -9, -13, -15 and -21. Each of these cytokines contains γc in its receptor complex, as well as a cytokine-specific α chain, and in the case of the IL-2R and IL-15R, a common β subunit known as IL-2Rβ (He and Malek, 1998). This sharing of receptor subunits allows different family members to mediate common downstream effects, a phenomenon termed cytokine redundancy (Lin et al., 1995). IL-7R signaling promotes the maintenance of cell survival by inducing a favorable balance of Bcl-2 family members (Brady et al., 1996; Chao and Korsmeyer, 1998). The Bcl-2 family represents a group of genes that function in antagonizing or agonizing apoptosis (Cory, 1995; Korsmeyer, 1995).

The predominant role of IL-7 in the DN stage includes the regulation of thymocyte differentiation (Peschon et al., 1994), proliferation (Conlon et al., 1989; Suda et al., 1990; Plum et al., 1993) and survival (Suda and Zlotnik, 1991; Kim et al., 1998).

In addition to IL-7, two other cytokines from the γc cytokine family, IL-2 and IL-15, seem to play a role in T cell development (Porter and Malek, 1999; Bassiri and Carding, 2001; Wrenshall et al., 2007), although their deficiency does not induce a drastic impairment in this process and therefore, their specific roles are still under investigation.

Once the T cell differentiation process in the thymus is finished, mature single positive thymocytes (either $CD4^+$ or $CD8^+$) leave this organ and seed the peripheral pool. This process starts around birth and continues throughout life.

The peripheral T cell pool may be divided into naive and activated/memory pools. The cells that constitute those two pools can be phenotypically and functionally distinguished. Lymphocytes leaving the thymus will fill mainly the naive T cell compartment, and thus this pool is characterized for having a big diversity of T cells. The activated/memory pool has lower T cell diversity, but has T cells characterized for having a much more efficient effector function.

2 PERIPHERAL NAIVE T CELL POOL

2.1 Naive T cell characterization

Naive T cells are phenotypically characterized by low levels of CD44 and Ly-6C (Curtsinger et al., 1998); high levels of CD62-L and CCR7 (in both $CD4^+$ and $CD8^+$ T cells) and high levels of CD45RB in the subpopulation of $CD4^+$ T cells (Berard and Tough, 2002). These cells have never been in contact with their cognate antigen. They are thought to be long-lived, relatively quiescent and rarely dividing (Tough and Sprent, 1994).

2.2 Homeostasis of Naive T cells

Thymus production in normal mice largely exceeds the quantitative requirements to replenish the number of T cells in the peripheral pools (Almeida et al., 2001). In these

circumstances, each newly produced T cell has to compete either with other newly produced cells or resident cells to survive in the periphery (Freitas and Rocha, 1993). There are two ways to leave the naive pool, either by death or by activation and differentiation of T cells (passing from the naive into the activated/memory pool).
The maintenance of naive T cells is mainly controlled by two types of resources:

2.2.1 The role of p-MHC

Several studies have shown that survival of both $CD4^+$ and $CD8^+$ T cells is an active process depending on TCR signaling. Two different strategies were used to study this requirement. One strategy relied on conditional TCR ablation, either by using a tetracycline based (Labrecque et al., 2001) or an inducible KO system (Polic et al., 2001). Both studies, using this type of approach, showed a progressive decay of the TCR deficient T cells. From these results two hypothesis could be raised: either cells need a continuous TCR signaling (meaning that the presence of the T cell receptor, for itself, induced a signaling) or T cells need to interact with p-MHC through the TCR. The second strategy, relying on the use of MHC deficient mice has permited to conclude that the second hypothesis was the correct one.
The first evidence that the survival of naive T cells requires TCR/p-MHC interactions came from the observation that $CD4^+$ T cells, coming out from an engrafted wild-type spleen in MHC class $II^{-/-}$ recipients, gradually decreased in number with time in the periphery (Takeda et al., 1996). Nevertheless there were some critics relative to Takeda's work, saying that the longer survival observed in the $CD4^+$ T cells could have been due to potential contamination of the MHC Class II deficient peripheral organs of the host with MHC Class II^+ donor type cells originated from the untreated transplanted fetal thymus. However, a study comparing the $CD4^+$ T cell survival between grafted treated thymus in MHC class II deficient mice and in mice that were MHC class II KO for all cells except the DCs (Brocker, 1997); and a study based on transplantation of treated thymus in MHC haplotype mice (Kirberg et al., 1997), showed similar results. To avoid surgical interventions in mice, two reports used the strategy of transient expression of class II in the thymus of class II deficient hosts either by using intrathymic delivery of recombinant adenovirus (Rooke et al., 1997) or tetracycline controllable class II expression in thymic epithelial cells (Witherden et al., 2000). Other reports did adoptive or BM transfers into either

haplotype mice (Boursalian and Bottomly, 1999) or into MHC classII KO (Ernst et al., 1999; Martin et al., 2003) mice. All these reports have demonstrated the need of TCR interaction with p-MHC for the survival of naive $CD4^+$ T cells.

For the $CD8^+$ T cells, wild type thymus engraftment in a MHC class I deficient mouse also revealed a reduction in the number of naive $CD8^+$ T cells in the periphery (Nesic and Vukmanovic, 1998). But the cleanest study done, that showed, without any doubts of possible MHC contamination, the need of p-MHC for naive $CD8^+$ T cell survival, results from adoptive transfer of naive aHY CD8+ T cells into MHC class $I^{-/-}$ hosts and the observation of T cell reduction with time (Tanchot et al., 1997). In both $CD8^+$ T studies, the rate of T cell reduction over time was faster comparing to the $CD4^+$ T cell studies. The faster $CD8^+$ T cell reduction comparing to $CD4^+$ T cells was also observed in the reports of TCR ablation (Labrecque et al., 2001; Polic et al., 2001); nevertheless in these studies, the overall T cell reduction rate was slower than in the T cell transfers into MHC deficient studies (Tanchot et al., 1997). A possible explanation for this difference may be related with the fact that after TCR ablation, the T cell receptors do not disappear 100% immediately and therefore, they are still able to receive signals through interaction with p-MHC; while in adoptive transfers into MHC KO mice, donor T cells will have an acute lack of TCR/p-MHC interaction and they will start to die faster.

But if all the reports mentioned above agree in the need of MHC class for survival of both $CD4^+$ and $CD8^+$ naive T cells, three other reports showed that the survival of naive $CD4^+$ T cells in MHC class $II^{-/-}$ vs MHC class II^+ hosts is quite similar (Clarke and Rudensky, 2000; Dorfman et al., 2000; Grandjean et al., 2003). However, it has to be pointed out that these reports used MHC class II-deficient mice that were I-$A\beta^{-/-}$, which are not totally MHC class II deficient because of the α chain of I-A that can dimerize with the I-E β chain. Moreover, the adoptively transferred naive $CD4^+$ T cells in these hosts likely have a higher amount of other resources (e.g. cytokines) than in lymphoreplete wt mice; this may compensate a lower MHC class II expression.

2.2.2 The role of cytokines

Other factors besides TCR tickling with p-MHC seem to be required for naive T cell survival in the periphery. The initial clue that cytokines support survival of naive T cells arose from the finding that certain cytokines, namely IL-4, IL-6 and IL-7, are able to keep naive T cells alive under *in vitro* conditions (Vella et al., 1997). For IL-6 there is no direct evidence that this cytokine is important for naive T cells survival *in vivo*. However, for the other two cytokines, the life span of naive $CD4^+$ T cells in thymectomized mice was reported to be severely shortened when contact with both IL-4 and IL-7 was blocked (Boursalian and Bottomly, 1999). Nonetheless, a more recent study (using a similar system but instead of TCR Tg $CD4^+$ T cells, they monitored a polyclonal $CD4^+$ T cell population and instead of injecting anti-IL-7, they injected anti-IL7R), has concluded that only IL-7 has a role in the survival of naive T cell in the periphery (Vivien et al., 2001). A possible explanation for the divergent results could be that a diverse set of cells may have different survival needs when compared to a more restricted population.

But the best evidence for the role of cytokines in survival comes from a report that showed that T cells from TCR Tg Marilyn $\gamma c^{-/-}$ mice (that have low T cell numbers in the periphery), when transferred into syngeneic $RAG2^{-/-}\gamma c^{-/-}$ hosts, were lost within 5 days following transfer, whereas the TCR Tg Marilyn γc^+ control survived upon transfer (Lantz et al., 2000). Although this result does not allow to discriminate which cytokine of the γc family is responsible for $CD4^+$ T cell survival, it supports the notion that at least one cytokine, probably IL-7, of this family has a crucial role in naive $CD4^+$ T cell survival in the periphery.

The importance of IL-7 for naive $CD8^+$ T cell survival was observed when the OT1 TCR Tg cells deficient for IL-7Rα chain did not survive more than a week after adoptive transfer into normal syngeneic hosts (Schluns et al., 2000). Similarly, when normal polyclonal $CD4^+$ and $CD8^+$ T cells are transferred into irradiated syngeneic $IL\text{-}7^{-/-}$ hosts, the naive T cells from both subsets disappear within one month (Tan et al., 2001).

Altogether it became clear that besides having an indispensable role in T cell development, IL-7 is also a very important requirement for the survival of the naive T cells in the periphery.

3 PERIPHERAL ACTIVATED/MEMORY T CELL POOL

3.1 Activated/Memory T cell characterization

T cells that constitute the activated/memory pool are phenotypically characterized for expressing higher levels of several adhesion molecules compared to naive T cells. In mice, the most common and reliable markers for activated/memory T cells is the high expression of CD44 for both $CD4^+$ and $CD8^+$ T cells, the low expression of CD45RB for the $CD4^+$ T cells and the high expression of Ly6-C for $CD8^+$ T cells (Berard and Tough, 2002). Although in many reports, based on this surface phenotype, refer to these cells as memory T cells, it should be noted that effector and memory-like cells share similar phenotypes.

The definition of memory cells appeared with the first immunization studies. The original definition is that they are long-lived T cells that survive upon clearance of their cognate antigen and that respond faster and more efficiently than naive T cells when re-exposed to the antigen that they had encountered previously. In other words, memory T cells proliferate faster and produce effector molecules (e.g. IFNγ) more strongly than naive T cells. This was first observed by *in vitro* (Dutton et al., 1998; Bachmann et al., 1999a; Zimmermann et al., 1999) and later by *in vivo* experiments (Garcia et al., 1999; Veiga-Fernandes et al., 2000). Several factors are responsible for this observation: memory T cells have less stringent requirements for activation than naive (e.g. they respond to lower concentration of antigens); they are less dependent on co-stimulatory signals; they do not require as long a duration of antigenic stimulation; and, in addition, memory T cells possess a higher content of both RNA and protein than naive T cells, suggesting that memory cells may be resting in the G1 rather than the G0 phase of the cell cycle (Stout and Suttles, 1992; Veiga-Fernandes et al., 2000; Berard and Tough, 2002).

Effector cells are cells that release cytokines or execute their cytolitic activity without the need for further differentiation. Those cells are the result of the activation of the naive or memory T cells.

Memory-like cells are defined as T cells that have a similar function and phenotype to memory cells, but which are originated from activation due to the “sense of empty space” in lymphopenic hosts (Murali-Krishna and Ahmed, 2000).

The pool of activated/memory T cells is a very heterogeneous pool. For example, among the $CD8^+$ $CD44^{hi}$ T cells, a percentage is $CD122^{lo}$, although the vast majority is $CD122^{hi}$ (Judge et al., 2002). Another example, among many others, is that in both $CD8^+$ and $CD4^+$ $CD44^{hi}$ T cells, there is a subset of cells that are CD62-L^+ $CCR7^+$ and another subset that are CD62-L^- $CCR7^-$. There is controversy over whether this memory T cell heterogeneity is a function of different stages in a linear differentiation pathway or whether it reflects plasticity in T cell antigenic and functional phenotype as a consequence of exposure to different types of antigenic stimuli within different microenvironments (Appay et al., 2002; Catalina et al., 2002; Roman et al., 2002; Baron et al., 2003; Wherry et al., 2003).

3.2 Activated/Memory T cell homeostasis

Like in the naive pool, the activated/memory T cell pool is subject to stringent homeostatic control mechanisms that integrate three processes: generation, survival and death of activated/memory cells.

The generation of activated/memory T cells results from the activation and differentiation of naive T cells. Classically, T cell activation occurs, for example, after infection (either with virus, bacteria or parasites) or tumor development, upon presentation of antigen by the antigen presenting cells (APCs) (Morrison et al., 1986; Germain, 1994), along with costimulation (Lenschow et al., 1996) and inflammatory signals (Harty and Badovinac, 2008).

Lymphopenia can be induced by radio- and chemotherapies used in cancer treatment and tissue engraftment. It can also occur as a consequence of infections with pathogens. An obvious example is acuired immunodeficiency syndrome, AIDS, in which T cell numbers are chronically reduced (Margolick and Donnenberg, 1997), but other viral diseases also induce transient, but quite marked T cell lymphopenia (e.g. influenza virus) (Okada et al., 2000; Tumpey et al., 2000). Although in some cases this lymphopenia may induce T cell proliferation, it is not known whether lymphopenia always leads to proliferation. There is also one situation of natural lymphopenia that occurs in very young individuals. In young mammals, the initial waves of T cells that leave the thymus migrate into the “empty” secondary lymphoid tissues. It was reported

that neonatal T cells entering the neonatal periphery, proliferated strongly, but in this particular case they exceptionally retained a naive phenotype (Le Campion, 2002 #107).

In summary, although many of the cells in the activated/memory pool are bona fide memory T cells that result from immune responses to environmental pathogens, some of them might have arisen through lymphopenia driven proliferation (LDP).

3.2.1 Generation of activated/memory T cells in the classical activation situation (infection)

Naive T cell responses to pathogen can be divided into four relatively distinct phases:

a) Initial activation
b) T-cell expansion and differentiation
c) Contraction
d) Memory phase

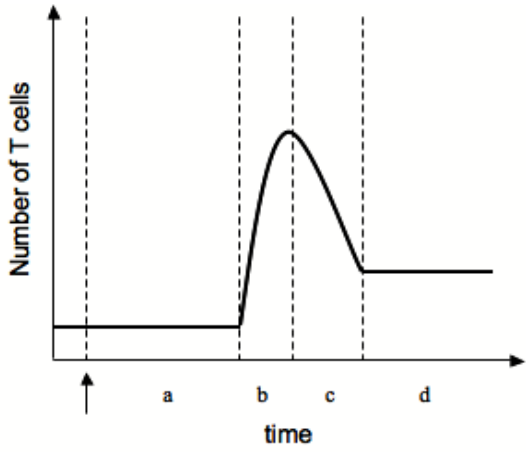


Figure 2: **Naive T cell immunization**. Upon challenge (indicated by the arrow), specific naive T cells will be activated (a) and they will expand and differentiate (b). When antigen wanes, most of T cells will die, and this is characterized as being the contraction phase (c). The cells that escape death will become memory T cells (d).

a) Initial activation

Primary activation of T cells involves the transmission of distinct inductive signals from antigen-loaded APCs to naive precursors in the T cell areas of the secondary lymphoid organs (spleen or lymph nodes). One important signal is antigen-specific and delivered via stimulation of the T cell antigen receptor (TCR) by peptide-MHC complexes on the APC surface (Morrison et al., 1986; Germain, 1994).

The co-stimulatory signal serves to amplify or modify the first signal by lowering the threshold required for responsiveness (Greenwald et al., 2005). B7-1 and B7-2 (also called CD80 and CD86 respectively), which are expressed on APCs, can transmit signals to naive T cells via CD28, which leads to recruitment of signaling molecules to the immunological synapse and downstream transcriptional activation of genes that promote T cell growth, differentiation and survival (Viola et al., 1999). The importance of B7-CD28 interactions is shown by the numerous defects in cellular immunity found in mice that lack these molecules (Greenwald et al., 2005). Other important receptors, expressed on T cells, responsible for co-stimulatory signaling are CD40, 41BB, CD27, ICOS and OX40 (Greenwald et al., 2005; Watts, 2005).

Another important signal is the inflammatory cytokines (e.g. IL-12, IFNα, IFNβ and IFNγ) that are important for inducing survival during proliferation and to induce differentiation of the naive T cells into robust effector cells (Haring et al., 2006). For the generation and later maintenance of memory $CD8^+$ T cells, it has been observed the additional requirement for $CD4^+$ helper T cells that recognize MHC class II restricted determinants presented on the same APC that is recognized by $CD8^+$ cytotoxic T lymphocytes (CTL) during their initial activation (van Stipdonk et al., 2001; Janssen et al., 2005). $CD4^+$ T cell help was thought to occur through the release of specific cytokines required for CTL differentiation, and through induction of functional maturation of the APC via interactions between CD40 and its ligand (Mackey et al., 1998). However, a more recent report has showed that $CD4^+$ T cell helps on generation of memory $CD8^+$ T cells through direct interactions of CD40-L of $CD4^+$ T cells with CD40 that is expressed on $CD8^+$ T cells (Bourgeois et al., 2002). The need of $CD4^+$ T cells was also supported by the observation that, in the absence of $CD4^+$ T cells during priming, $CD8^+$ T cells exhibited heightened expression of the death receptor TRAIL (tumor-necrosis factor related apoptosis-inducing ligand), and underwent apoptosis when re-stimulated (van Stipdonk et al., 2001; Janssen et al., 2005).

b) T-cell expansion and differentiation

T-cell differentiation involves the regulation of transcriptional programs that control the cell cycle, response to cytokines, migratory capacity, effector function (cytokines secretion, cytotoxicity) and susceptibility to activation induced cell death (AICD). Although $CD4^+$ and $CD8^+$ T cells both commit to proliferation after antigenic stimulation, $CD4^+$ T cells have a slower rate of cell division *in vitro* and *in vivo* compared with $CD8^+$ T cells (Maini et al., 2000; Homann et al., 2001). In addition, the increase in the number of antigen specific $CD8^+$ T cells is substantially greater than that of $CD4^+$ T cells during viral and bacterial infections in mice (Murali-Krishna et al., 1998; Homann et al., 2001; Foulds et al., 2002), despite some exceptions, such as the case of Salmonella (Srinivasan et al., 2004).

$CD8^+$ T cell differentiation

For $CD8^+$ T cells, the differentiation phase is linked to an extensive proliferation that is promoted by cytokines such as IL-2 (Cheng et al., 2002; Kamimura and Bevan, 2007), IL-12 (Curtsinger et al., 1999), type I IFNs (Curtsinger et al., 2005) and IFNγ (Whitmire et al., 2005) together with the acquisition of cytolytic activity (perforin and granzyme production), the production of IFNγ and TNFα and migratory capacities (acquisition and/or loss of certain chemokine receptors) (Haring et al., 2006).

$CD4^+$ T cell differentiation

Depending on the cytokine milieu, $CD4^+$ T cells can be polarized into at least 3 different helper subsets: Th1 (induced by IL-12 and produces IFNγ), Th2 (polarized by IL-4 and also releases IL-4) (Murphy and Reiner, 2002) and Th17 cells (polarized by IL-6 along with TGF-β and secretes IL-17) (Stockinger and Veldhoen, 2007). Although certainly less caricaturated than this, each subset is thought to have a define role in fighting against microorganisms. Th1 T cells are involved in the fight against viral infections, by inducing the cytolitic action of $CD8^+$ T cells (Kalinski and Moser, 2005); Th2 are involved in combating bacterial pathogens, by stimulating B cell activation and inducing therefore the release of antibodies (Kalinski and Moser, 2005). And Th17 cells have an important role in inducing neutrophil-mediated protective immune response

against extracellular bacterial or fungal pathogens such as *Klebsiella pneumoniae* (Happel et al., 2003; Happel et al., 2005) and *Candida albicans* (Huang et al., 2004).

c) Contraction

In an acute infection, the clearance of antigen is accompanied by the shutdown of the T cell immune responses and involves apoptosis of a large fraction of antigen-activated T cells. This phenomenon is called activation induced cell death (AICD). It is believed that a decrease in growth factors following pathogen clearance governs the onset of contraction (Sprent and Tough, 2001). Candidate factors include type I interferons (IFNs) and members of the IL-2 family (i.e.) IL-2, IL-4, IL-7 and IL-15. However, other data suggest that this phase may be programmed early in the response (Badovinac et al., 2002). This apoptosis prevents accumulation of no-longer-needed and potentially dangerous effector cells and thereby avoids immunopathology.

In viral infections, while around 90-95% of $CD8^+$ T cells die of apoptosis in a 1-2 week period, approximately 50 days are required to remove a similar percentage of activated $CD4^+$ T cells (Murali-Krishna et al., 1998; Homann et al., 2001).

It was reported that the expression of the transcriptional repressor Blimp-1 is essential in the contraction phase. Loss of Blimp-1 function either by doing experiments with a knock-in GFP mouse that substitutes Prdm1 gene that encondes Blimp-1, (Kallies et al., 2006); or with a conditional knock-out mouse, that resulted from crossing $Prdm1^{flox/flox}$ with mice expressing a Cre transgene under control of the Lck proximal promoter (Martins et al., 2006), revealed accumulation of T cells (Kallies et al., 2006; Martins et al., 2006). However, besides the importance of the upregulation of certain genes during this phase, repression of a different set of genes is also necessary for contraction to occur. One known example is the case of the Immediate Early response gene X-1 (IEX-1). It was shown that IEX-1 is highly expressed at early stages of activation, but declines with a prolonged period of activation time, coincident with the contraction period. Constitutive expression of this gene impairs apoptosis and induces a lupus-like autoimmune disease (Zhang et al., 2002)

Two distinct apoptotic cell-death pathways that ultimately converge at the executioner caspases (caspases 3 and 9) are known in mammalian cells (Strasser et al., 1995):

-The intrinsic pathway is mediated by the mitochondria and functions in response to certain stimuli such as cytokine deprivation, reactive oxygen species (ROS), DNA damage and calcium overload. The pro-apoptotic and anti-apoptotic members of the Bcl-2 superfamily tightly regulate this pathway. Pro-apoptotic family members include Bax, Bak, and Bok in addition to the BH3-only proteins Bik, Bid, Bad, Bmf, Hrk, Noxa and Puma. The anti-apoptotic family members include Bcl-2, Bcl-xL, Bcl-w, Mcl-1 and A1 (Strasser, 2005). This apoptotic pathway is characterized by the release of cytochrome c from the mitochondria. In the apoptosome, cytochrome c, APAF-1, dATP and pro-caspase 9 oligomerize, leading to the generation of active caspase 9 (Li et al., 1997). This enzyme continues the apoptotic cascade by activating downstream executioner caspases such as caspases 3 and 7.

-The extrinsic pathway is mediated by death receptors interacting with their ligand. There are several death receptors including Fas, tumor necrosis factor receptor (TNFR) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Fas et al., 2006). After ligation, the death-inducing signaling complex (DISC) is formed and it recruits pro-caspase 8 and causes its activation (Muzio et al., 1996). Caspase 8 then activates executioner caspases such caspases 3 and 7, resulting in apoptosis.

The relative contributions of the two distinct apoptotic pathways in the termination of T cell immune responses has been a matter of controversy for a long time because some studies ascribe that function to the extrinsic pathway, through Fas/FasL interactions (Russell et al., 1993; Brunner et al., 1995), while others attribute that function to the intrinsic pathway, because of the relevant role of Bcl-2 (Hildeman et al., 2002) Bcl-xl (Garcia et al., 1999), Bax and Bak (Rathmell et al., 2002). These discrepancies might be explained, at least for $CD8^+$ T cells, by a recent paper that shows that in acute immune responses, death is mediated predominantly by the intrinsic pathway. However, both the extrinsic and intrinsic apoptotic pathways play a role during chronic antigen stimulation, to prevent deregulated expansion of activated T cells (Hughes et al., 2008)

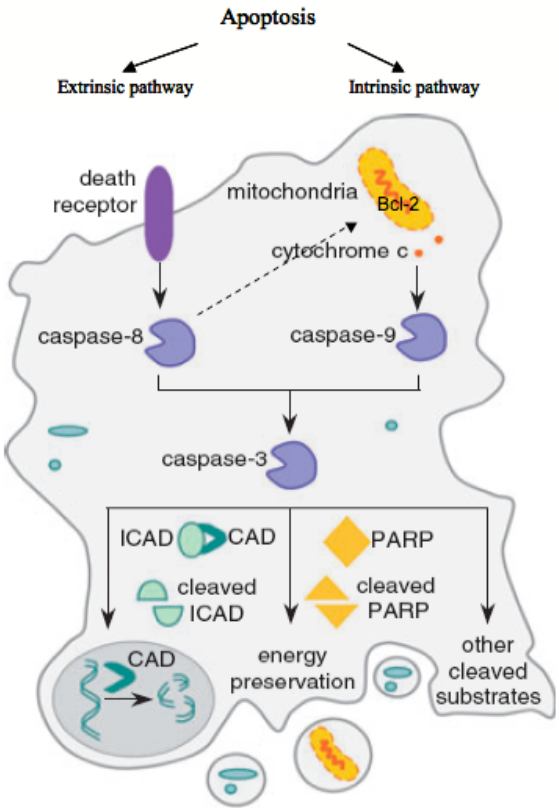


Figure 3: **Apoptotic pathways**. Scheme showing either the extrinsic apoptotic pathway or the intrinsic apoptotic pathway (adapted from Fink and Cookson, 2007).

d) Memory phase

After the proliferative burst that is characteristic of the primary T cell response, most effector T cells disappear, but a minor fraction of cells persist as long-term memory T cells. Generation of memory T cells has been and it is a controversial subject. There are two general and mutually exclusive models: the non-linear differentiation model and the linear differentiation model. The non-linear model proposes that memory T cell development occurs independently of effector function during the initial priming phase. Some reports have shown that T cells that expressed activation markers and that proliferated, but didn't produce cytokines or other effector molecules, were the ones that gave rise to memory T cells (Lauvau et al., 2001; Wu et al., 2002a). In contrary, the linear model proposes the sequential differentiation of naive to effector to

memory T cells (Jacob and Baltimore, 1999; Harrington et al., 2008). Within the linear model, many variants have been proposed to account for variations in activation during priming: the progressive differentiation model (Lanzavecchia and Sallusto, 2002) and the instructive/programmed differentiation model (Kaech and Ahmed, 2001).

The progressive-differentiation model

The progressive-differentiation model defends that T-cell fate depends on the signal strength that they receive by interacting with APCs. There would be 3 factors intrinsic to APC that determine the amount of signal: the number of APCs, the concentration of peptide-MHC complexes and the concentration of co-stimulatory molecules that will be responsible for the duration of the interaction between T cells and APCs. This model proposes that memory T cells are generated when one or more signals necessary for terminal differentiation into effector cells are lacking, as antigen and infection are waning (Lanzavecchia and Sallusto, 2002). Therefore, memory cells arise from precursors that are not fully differentiated effector cells. This model has been supported by recent papers, which show that latecomers $CD8^+$ (van Faassen et al., 2005) and $CD4^+$ T (Jelley-Gibbs et al., 2005; Catron et al., 2006) T cells exhibit enhanced memory formation. However, there is also a recent report showing that while latecomer $CD8^+$ T cells have a unique differentiation program, those cells are not preferentially recruited into the memory pool (D'Souza and Hedrick, 2006).

The instructive/programmed differentiation model

The instructive/programmed differentiation model predicts that a brief encounter with peptide-MHC on an APC is sufficient for naive T cells to set motion a program of proliferation and differentiation, where memory cells arise directly from the few surviving effector cells that evade AICD after antigen stimulation. This model was initially based on the finding that activating $CD8^+$ P14 T cell *in vitro* for 24 hours and injecting them subsequently into a B6 wt mouse, those cells are able to differentiate into effectors and then functional memory T cells (Kaech and Ahmed, 2001). In favor of this model, another report also showed that memory $CD8^+$ OT1 T cells can be generated *in vivo* as early as 3.5 days after activation (Kedzierska et al., 2007).

Nevertheless this model has some deficiencies. On the one hand, up to now, the early-programmed proliferation has only been shown for $CD8^+$ T cells (Mercado et al., 2000; Kaech and Ahmed, 2001; van Stipdonk et al., 2001; Wong and Pamer, 2001; Kedzierska et al., 2007). On the other hand, even if Kaech et al have observed a synchronous and extensive cell division, it cannot be determined whether all the cells received the same signal strength. Perhaps there are signals being received by responding cells that will be responsible for a different final outcome for those effector cells. Therefore, even if for the $CD8^+$ T cells they observe that a brief contact with antigen (as short as 24 hours *in vitro*) is sufficient to induce differentiation, that does not contradict with the progressive-differentiation model, because a high level of stimulation can be delivered in a relatively short time if the levels of antigen and co-stimulation are high enough and if the TCR has a particularly high avidity.

In the end, these two models are not mutually exclusive. Maybe the controversy comes from the fact that the word "progressive" differentiation induces to think that it takes time for naive cells to give rise to memory T cell precursors, when in fact it depends on the strength of the signal that a naive T cell receives and the signal strengths received by the expanding and differentiating populations. The corresponding signal strengths will depend on the type of immunization or pathogen infection. To avoid this potential misunderstanding, the comprehensive model of memory T cell generation should be named "signal-strength differentiation" model.

Recently it has been proposed a model that tries to integrate all the data and that was named intersecting pathway model (Moulton and Farber, 2006). In this model, cells that have been only activated (without acquiring effector functions) and effector cells that haven't reached the terminal differentiation, both can give rise to memory T cells (Moulton et al., 2006).

Subsets of memory T cells and models of their differentiation

Two types of memory T cells have been described in $CD4^+$ and $CD8^+$ T cell populations based on their anatomical location, expression of cell surface markers and effector functions (Sallusto et al., 1999; Masopust et al., 2001; Reinhardt et al., 2001). Memory T cells that express molecules such CD62L and CCR7, which allow efficient homing to lymph nodes (LNs), are termed central memory T cells (T_{CM}), whereas memory T cells that lack expression of these LN

homing receptors and are located in non lymphoid tissues are termed effector memory T cells (T_{EM}). However, both T cell subsets are present in the blood and spleen. These studies have also shown, that T_{EM} acquire effector functions, such as cytokine production and killing, more rapidly than T_{CM}, but, T_{CM} have an inherent proliferative advantage over T_{EM} following antigen stimulation (Wherry et al., 2003). However, others studies consider that these two subsets do not differ in immediate effector cell function (Champagne et al., 2001; Unsoeld et al., 2002).
The existence of these memory subsets raised the question of how T_{CM} and T_{EM} cells are generated and whether each is the product of interdependent or separate lineages. Three models of differentiation have been suggested:

-The **first** proposes a linear differentiation model in which T_{CM} differentiate to T_{EM}. This model was first based on the findings that memory human $CCR7^+$ T cells in short-term *in vitro* culture can lose expression of this chemokine receptor, and in the process become functionally competent (Sallusto et al., 1999). Recently, an *in vivo* study, in mice, also concluded that T_{CM} could be converted into T_{EM} upon *Listeria monocytogenes* infection, but the reverse was not possible (Huster et al., 2006).
-The **second,** like the first model, also proposes a linear differentiation model, but in an inverted way. This model considers that naive T cells become effector cells that then differentiate first into T_{EM} and that over time this subset converts into T_{CM} cells (Wherry et al., 2003). The first evidence for this differentiation was the fact that after clearance of the pathogen lymphocytic choriomeningitis virus (LCMV), although the total number of gp33-specific memory T cells in the spleen remained constant between 1 and 3 months, during this time the absolute number of T_{EM} decreased whereas the number of T_{CM} increased proportionally. But to be sure that was conversion of T_{EM} to T_{CM}, they sorted these two subsets of memory T cells and they transferred them into naive recipients. After 25 days, the transferred T_{CM} remained uniformly $CD62L^{hi}$, but half of the transferred T_{EM} cells had converted to $CD62L^{hi}$.
-The **third** considers that T_{CM} and T_{EM} cells represent mostly separate lineages (Baron et al., 2003). This study was done by analyzing the T cell receptor (TCR) repertoire of human blood memory $CD8^+$ T cells and finding that the T_{CM} and T_{EM} compartments have substantially different TCR repertoires. This has been interpreted as support for recruitment from distinct naive precursor cells.

Despite the fact that each of these 3 models have been proposed based on experimental results, the "theory" subjacent to each model is mutually exclusive from the other two. For this reason, intensive investigation has been developed around this thematic, in an attempt to unravel the memory T cell subsets relationship, as it is of great interest, especially improved future vaccination strategies.

The first model can be easily refuted because the observed conversion of T_{CM} into T_{EM} only occurred upon stimulation with the nominal antigen. Therefore, instead of looking at T_{EM} cells, they were observing the activation of memory T cells, upon re-stimulation, and the formation of effector T cells.

The second model has also been questioned by the fact that it was demonstrated that naive T cell frequency profoundly influences the pathway along which memory $CD8^+$ T cells develop (Marzo et al., 2005): whereas at high precursor frequencies, the T_{EM} converted into T_{CM}, at low precursor frequencies, the T_{EM} generated cells represented a stable cell lineage that failed to further differentiate into $T_{CM.}$ More recent reports have also shown that initial T cell frequency affects both effector and/or memory generation, either by using adoptive transfer of TCR Tg cells (Badovinac et al., 2007) or by studying endogenous naive $CD8^+$ T cells fate upon infection (Obar et al., 2008). However, the last report (Obar et al., 2008) claims that although higher endogenous precursor frequencies converted from T_{EM} to T_{CM}, lower-frequency endogenous T cell also converted toward T_{CM}. The only difference is that conversion takes longer. Nevertheless, they look at the percentage of CD62-L^{hi} cells (and not numbers) without doing a comparative study with the fate of CD62-L^{lo} T cells. Therefore, we cannot be sure, in this case, if we are in presence of T_{EM} to T_{CM} conversion.

The arguments that supported the third model were also refuted by the fact that it was found individual clonotypes of epitope-specific TCRs in different subsets (Bouneaud et al., 2005) and that a single naive $CD8^+$ T cell precursor can develop into diverse effector and memory subsets (Stemberger et al., 2007).

In the end, these results lead to the hypothesis that actually there is no two memory subsets, but only one. We could imagine that the T_{EM} are not a subset of memory T cells, but are actually real effector cells. For example, recent papers have shown that although there is no influenza virus detection in the lungs and in other tissues after day 12 of infection (either by PFU counting or by a sensitive PCR-based test), there might exist an unexpected prolonged

presentation of influenza antigens that promotes an activated phenotype of both $CD4^+$ (Jelley-Gibbs et al., 2005) and $CD8^+$ T cells (Zammit et al., 2006). The observations for the $CD4^+$ T cells were done by transferring naive $CD4^+$ T cells into mice, that had supposedly cleared virus, and realizing that those cells divided and migrated to different organs, including the lungs, and were $CD62L^{lo}$. For the $CD8^+$ T cells, the same type of conclusion came up from parabiosis studies, with mice that had cleared the infection and with non-infected mice. The conclusion resulted from the study of the migration and activation pattern of $CD8^+$ T cells originated from the two different mice. In addition to the finding of virus persistence even when it's assumed that it was eliminated by the immune response (acute infection) (Ciurea et al., 1999), the existence in lymphoid tissues of structures adapted for long-term maintenance of antigen has also been reported. The antigen is stored for many months and possibly years, in the form of immune complexes, on the surface of follicular dendritic cells (FDCs) (Tew and Mandel, 1979). These findings point to the notion that T_{EM} are not really memory cells but effector cells that are either long-lived or continuously produced upon stochastic contact of memory T cells with FDCs, for example, or with other cells that present the antigen. Thus, following this line of thinking, we can say that the real memory cells are the ones that are usually referred as T_{CM}. An important observation shown by different reports that supports this hypothesis is that protective immunity (either against virus or bacteria) is more efficiently conferred by T_{CM} than T_{EM}, due to the greater proliferative capacity of T_{CM} (Catron et al., 2006; Wherry et al., 2003). Therefore T_{CM} seem to be the only ones that have acquired the two-hallmark characteristics of memory T cells: ability to mount secondary responses and the stem-cell-like quality of self-renewal.

Molecular signature of memory T cells

On the one hand, it has been shown that naive, effector and memory $CD8^+$ T cells have a unique and different molecular signature based on transcriptional profiling (Kaech et al., 2002). On the other hand it was shown that, in the mouse, a portion of the genetic program of hematopoietic stem cells is shared with embryonic and neural stem cells (Ivanova et al., 2002). In other words, there is a stem cell molecular signature. Because it is suggested that memory T cells have a stem-cell-like quality of self-renewal, a group decided to compare the gene-expression profiles of naive, effector and memory $CD8^+$ T cells with those of long-term hematopoietic stem cells (Lt-HSC), short-term hematopoietic stem cells (St-HSC), and lineage committed progenitors

(LCP) (Luckey et al., 2006). What they found was that transcripts augmented in memory $CD8^+$ T cells relative to naive and effector T cells were selectevely enriched in Lt-HSC and were progressive lost in their short-term and lineage-committed counterparts. And transcripts selectively decreased in memory T cells were also selectively decreased in Lt-HSP and progressively augmented with differentiation. To test whether that T_{CM} are the only real memory T cells, one could perform a molecular study comparing the transcripts between Lt-HSC, T_{CM} and T_{EM}, to determine if there are differences in the transcripts between the currently accepted memory subsets, and to determine if there is a higher similarity between Lt-HSC and T_{CM} or between Lt-HSC and T_{EM}.

Markers to identify activated cells that will become memory cells

Independently of whether there are one or two types of memory T cells, there have been extensive studies to try to find out the marker(s) that identify the activated cells that are potential future memory cells.

The first evidence that high level expression of IL-7Rα (also named CD127) could be a marker for precursor memory T cells came from a study that showed that mice infected 3 weeks after transfer of $CD127^{hi}$ $CD8^+$ $P14^+$ effector T cells, were provided of immunological protection. In contrast, the same approach but with transfer of $CD127^{lo}$ $CD8^+$ $P14^+$ effector T cells, didn't confer protection (Kaech et al., 2003). A more recent study add that $CD127^{hi}$ $KLRG1^{lo}$ expression along with low expression of T-bet promoted the differentiation of effector cells into memory precursors (Joshi et al., 2007). This observation comes from the use of two different strategies. One consisted in the Tbx21 gene dosage (Tbx21 is the gene of T-bet) by comparing $Tbx21^{+/+}$, $Tbx21^{+/-}$ and $Tbx21^{-/-}$ P14 $CD8^+$ T cells during LCMV infection; and the other consisted in transfecting $Tbx21^{-/-}$ P14 T cells with T-bet/estrogen receptor (ER) MSCV vector and transfer them into a mice that were later infected with LCMV and subsequently treated with different doses of tamoxifen (an estrogen). However, it is still not completely clear how absolute a role of IL-7Rα expression plays in selecting memory $CD8^+$ T cell precursors, because it has been demonstrated that IL-7Rα^+ cells also die during the contraction phase, by comparing the CD127 MFI with the apoptotic and non-apoptotic cells, i.e. annexin V^+ annexin V^- (Lacombe et al., 2005). Moreover, recent reports using constitutive expression of IL-7Rα in T cells show that expression of IL-7Rα alone does not support increased survival of both effector $CD4^+$ (Haring et

al., 2008) and $CD8^+$ T cells (Hand et al., 2007; Haring et al., 2008) into the memory phase following bacterial or viral infection. However, Kaech's group showed that expression of IL-7Rα is necessary, although not sufficient for memory generation: if mice are treated with rIL-7 during the contraction phase, there is an enhancement of IL-7Rα Tg effector P14 T cells compared to wt P14 T cells. However, they argue that overexpression of IL-7Rα does not rescue $KLRG1^{hi}$ effector cells from death because $KLRG1^{hi}$ IL-7Rα Tg effector $CD8^+$ T cells exhibit decrease responsiveness to IL-7 in vivo. (Hand et al., 2007).

Another report shows that Serine protease inhibitor 2A (Spi2A), an inhibitor of lysosomal executioner proteases dependent on the transcription factor NF-κB, promotes the survival of cytotoxic T lymphocytes, allowing them to differentiate into memory $CD8^+$ T cells (Liu et al., 2004). This was observed by using transfected BM chimeras with MIGR1 retrovirus, which expressed Spi2A, Spi2A antisense or GFP alone.

In this study they also observed that Spi2A correlates, not only with the differentiation of the effectors into memory $CD8^+$ T cells, but also with the expression of CD127.

Expression of the homodimer CD8αα in some effector $CD8^+$ T cells has also been described as a memory differentiation marker, by showing that CD8α enhancer-deficient mice ($E8I^{-/-}$) are compromised in generating memory $CD8^+$ T cells (Madakamutil et al., 2004). But a more recent report, making use of $E8I^{-/-}$ mice as well, demonstrates that generation of memory T cells can occur without the expression of CD8αα (Chandele and Kaech, 2005).

In spite the numerous attempts in trying to discover a reliable marker of precursor memory T cells, up to now, nobody has succeeded in finding it. However, although not perfect, it seems that IL-7Rα re-expression is one of the best markers for memory precursor cells.

In our work we will generate memory T cells by using protocols where we know that nominal antigen will totally disappear and where functional tests show that they proliferate and respond faster upon re-challenge.

3.2.2 Generation of activated/memory T cells in lymphopenic environments (LDP)

Lymphopenia driven proliferation (LDP) of peripheral T cells was first described either in thymectomized mice during T cell recovery following T cell elimination (Rocha et al., 1983) or following the fate of T cells adoptively transferred into T cell deficient hosts (Miller and Stutman, 1984; Freitas et al., 1986; Rocha et al., 1989; Rocha, 1987). The observation of a cellular expansion in response to empty space, led several groups to consider that this phenomenon should allow T cells to re-conquer the empty space and reestablish the number of cells observed in a normal individual. For this reason they named this phenomenon as homeostatic proliferation. But actually this proliferation does not reach the number of T cells observed in a normal mouse or human and the naive T cells acquire an activated/memory phenotype while proliferating, reducing therefore, the number of T cells in the naive pool (Mackall et al., 1997). Furthermore, not all T cells have the same potential to expand after adoptive transfer into T cell deficient mice, resulting in a repertoire that is limited in diversity and prone to skewing (Mackall et al., 1996; La Gruta et al., 2000). This skewing depends also on the intial number of cells injected: lower donor T cell numbers injected will induce a more limited repertoire than higher polyclonal T cell numbers injected (Min et al., 2004).

All these findings revealed that it is not correct to call this a homeostatic phenomenon. And despite the fact that many groups still use this designation (Jameson, 2002; Williams et al., 2007), it is more appropriate to call this phenomenon lymphopenia driven proliferation (LDP) or lymphopenia induced proliferation (LIP).

Generation of activated/memory T cells in lymphopenic environments can be divided into three distinct phases:

a) Initial activation

b) T cell proliferation/differentiation

c) memory-like phase

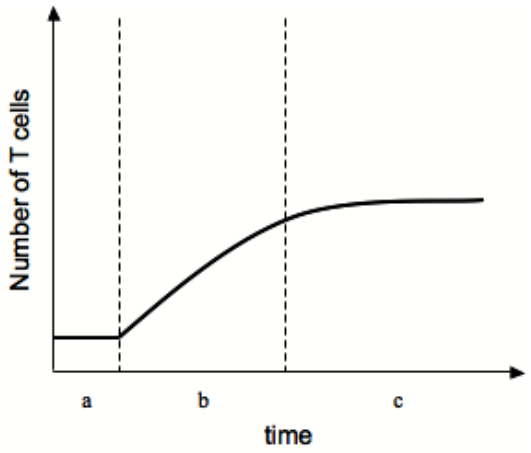


Figure 4: **Naive T cell LDP**. Upon transfer of naive T cells into a lymphopenic host, there is a period of initial activation (a), followed by T cell proliferation and differentiation (b), till the cells reach a plateau (c). When they reach the plateau those cells are considered as being memory-like T cells.

a) Initial activation

TCR/p-MHC interactions

Several results indicate that LDP requires TCR triggering. On the one hand, the disruption of the proximal TCR signaling through deletion of the p56 Lck kinase impairs LDP of naive T cells (Seddon and Zamoyska, 2002); on the other hand, conditional inactivation of the CD4 co-receptor abrogates the LDP (Wang et al., 2001), reinforcing the importance of the TCR/p-MHC interaction in this process. In addition, the different TCR Tg T cell readouts upon co-transfers into lymphopenic hosts revealed also the importance TCR/p-MHC interactions for LDP (Kassiotis et al., 2003; Kieper et al., 2004). Although these reports have referred that TCR affinity and/or avidity has an important role for $CD8^+$ (Kieper et al., 2004) and $CD4^+$ (Kassiotis et al., 2003) T cells to compete for LDP; in reality, the different outcomes of different TCR Tg T cells rely on the promiscuity of the T cells, as it was shown by Yi et al, and not on the affinity or avidity. But what is important from these studies is that they highlight, using competition experiments, the importance of TCR/p-MHC interactions for LDP. However, two studies with $CD4^+$ T cells demonstrated that the division of, at least, some of those cells could occur in the absence of MHC class II (Bender et al., 1999; Clarke and Rudensky, 2000). Nevertheless, like it

was already mentioned for naive T cell survival, these two reports used mice that, indeed, are not 100% MHC class II KO, and this may be the reason for the observed proliferation of some cells. Furthermore, very promiscuous TCR T cells might use non-classical MHC, as substitutive resources.

Two reports have shown that the source of antigens that drive LDP can come from either self peptides or from commensal bacteria in the intestines, and that the origin of the type of antigen regulates the LDP by different mechanisms for both $CD8^+$ (Kieper et al., 2005) and $CD4^+$ T cells (Min et al., 2005). We will mention later what are the different LDP stimuli.

Co-stimulatory molecules

One study has shown that LDP occurs independently of co-stimulation signals mediated through CD28/B7, or 4-1BB/4-1BBL interactions (Prlic et al., 2001). They concluded that by transferring either $CD4^+$ or $CD8^+$ T cells from wild type or $CD28^{-/-}$ mice into irradiated B6, or B6 4-$1BBL^{-/-}$ hosts and observing that the division pattern was similar independently of the situation. However, a more recent study, by also using $CD28^{-/-}$ mice, showed that CD28 has a role in the LDP of $CD4^+$ T cells (Hagen et al., 2004). Like in the previous report, a similar division patter was observed in both $CD28^{-/-}$ and wt $CD4^+$ T cells transferred into irradiated mice. Nevertheless, co-transfer of $CD28^{-/-}$ and wt $CD4^+$ T cells revealed that wt T cells had a competitive advantage over $CD28^{-/-}$ cells.

Altogether, we can conclude that when we have a diverse pool of T cells ongoing LDP, expression of co-stimulatory T cells can be of extremely importance. Cells with higher expression of co-stimulatory molecules have a better fitness than the others.

Cytokines

The importance of IL-7 for the occurrence of LDP was demonstrated either by observing that transfer of T cells into lymphopenic mice Tg for IL-7 ($CD3\varepsilon^{-/-}$) promoted proliferation of both polyclonal $CD4^+$ and $CD8^+$ T cells; or by observing an inhibition of cell division of either polyclonal (Bosco et al., 2005) or TCR Tg T cells when transferred into IL-$7^{-/-}$ recipients (Schluns et al., 2000; Tan et al., 2001); or by observing an impairment of polyclonal T cell division in lymphopenic mice that were treated with anti-IL7Rα mAb (Goldrath et al., 2002). Moreover, the fact that IL-7/IL-7 mAb complexes induce proliferation even in the absence of

lymphopenia, reflects that when this cytokine exists in non-limiting conditions (such as in a lymphopenic hosts), it is an important inducer of T expansion (Boyman et al., 2008).

More recent reports, by looking at the division pattern of transferred T cells into lymphopenic mice, IL-7 deficient or not (Kieper et al., 2005); or into lymphopenic mice treated with IL-7Rα monoclonal anyibody (Min et al., 2005), claimed that there are two subsets of LDP T cells: one that proliferates fast and is IL-7 independent, and the other that proliferates slow and is IL-7 dependent. One of these reports defends that the role of IL-7 on LDP depends on which kind of antigen sets off the division (Kieper et al., 2005), however there is no direct evidence supporting this suggestion.

Although initial experiments using T cell transfers into IL-4 and IL-15 KO mice have ruled out a role for IL-4 and IL-15 in LDP of naive T cells (Tan et al., 2001; Goldrath et al., 2002), a recent report, using the same strategy, shows that IL-15 is important not for initiation, but for sustained LDP and accumulation of $CD8^+$ T cells (Sandau et al., 2007). Recently, it has been shown the importance of IL-2 in driving naive $CD8^+$ T cell proliferation in lymphopenic hosts, by observing that while wt donor T cells strongly proliferated when transferred into $CD25^{-/-}$ mice; proliferation of $CD25^{-/-}$ donor T cells was undetectable (Cho et al., 2007). This suggested, that proliferation was selectively driven by raising levels of IL-2. This suggestion was further confirmed by experiments using rIL-2/αIL-2mAb.

b) T cell proliferation/ differentiation

Transfer of donor T cells into "natural" lymphopenic hosts (meaning, not submitted to any kind of treatment), results in two T cell proliferation patterns: one subset that proliferates fast and another subset that proliferates slowly. In contrast, transfer of donor T cells in irradiated, gnotobiotic and/or antibiotic treated lymphopenic mice, results only in the observation of a slow proliferation pattern (Kieper et al., 2005; Min et al., 2005). Therefore, these reports suggested that the fast rate of proliferation was related with the presence of commensal bacteria antigens, whereas the slow rate of proliferation was only due to sp-MHC. The importance of enteric bacteria is confirmed by the fact that inflammatory bowel disease (IBD) cannot be induced if the lymphopenic hosts are reared under germ-free conditions (Rath et al., 2001; Jiang et al., 2002).

Nevertheless, the dogma that there is a difference on the naive T cell proliferation rate depending on the antigen type of stimulation can also be questioned, at least for naive $CD8^+$ T cells, since the usual slow proliferation of naive $CD8^+$ T cells stimulated by sp antigens can be transformed into an intensive division upon IL-2 stimulation (Cho et al., 2007). However, a constant feature in the proliferation is that in both intentional immunization or in lymphopenia (independently of the antigen stimulus), the rate of proliferation of naive $CD4^+$ T cells is usually slower than $CD8^+$ T cells (Tan et al., 2001), even with IL-2 treatment (Cho et al., 2007).

Some reports claimed that up-regulation of activation markers (CD69, CD25) and/or the acquisition of effector functions during proliferation do not occur in irradiated mice, meaning in the absence of commensal bacterial (Goldrath and Bevan, 1999; Murali-Krishna and Ahmed, 2000; Kieper et al., 2005; Min et al., 2005). However, another report showed in irradiated mice that proliferating T cells could, indeed, express surface activation markers and/or effector functions, such as cytolytic activity (Oehen and Brduscha-Riem, 1999). Effector markers and effector function are observed upon transfer of T cells into non-irradiated lymphopenic mice (unpublished data).

Altogether, one can summarize that transfer of T cells into a lymphopenic host results in proliferation of the donor T cells, and in their differentiation into effector cells, at least in lymphodeficient mice with an intestinal flora.

c) memory-like phase

In contrary to “classical” activation /intentional immunization (figure 2), in LDP there is no contraction phase (figure 4) of T cells (instead they reach a plateau) and there is no antigen disappearance. The LDP of naive T cells occurs due to the sensing of space surrounding them, but the mechanisms that stop this proliferation are still debated. At least two general models have been proposed. The first suggests proliferation inhibition by physical T-cell-T cell interactions (similar to contact inhibition). Therefore, T cells would proliferate while contact inhibition is absent. Supporting this model is the fact that co-transfer of a large number of bystander T cells inhibits T cell proliferation in lymphopenic hosts (Ernst et al., 1999). The second model hypothesizes that T cells stop dividing when resources become limited and therefore they have to start competing for those limiting resources. Regarding this possibility, potential limiting

resources might include cytokines, namely IL-7 or IL-15, and access to APCs that present suitable self-peptide and/or intestinal flora antigens in their MHC complexes. Neither model has been conclusively ruled in or out yet, but there is experimental evidence demonstrating that T cells can indeed compete for resources. Over-expression of IL-7 increases the size of the T cell pool and favors LDP, which indicates that the levels of IL-7 are limiting in a normal host (Kieper et al., 2002). Another report, from our laboratory, that highlights the T cell competition for p-MHC by showing that reducing the number of cells expressing MHC class I will induce a reduction in the number of adoptively transferred monoclonal $CD8^+$ T cells when they reach the plateau (Hao et al., 2006).

LDP derived T cells acquire a phenotype and a function similar to the memory cells obtained from a conventional activation (Lin et al., 2008). This similarity was also observed at the molecular level, by comparing gene expression profiles of real memory and LDP-derived memory cells (Goldrath et al., 2004). However, a report has demonstrated that despite the similarities, real memory T cells are better competitors than LDP-derived memory cells; also named memory-like T cells (Bourgeois et al., 2005). Should be mentioned that a restricted memory-like T cell repertoire results from LDP (La Gruta et al., 2000) because not all the intially transferred cells are able to proliferate and survive long-term. It was shown that an average of 1 in 100 cells is able to expand (Rocha et al., 1989).

3.2.3 Survival/maintenance of memory T cells

a) The role of p-MHC

Initially, it was suggested that nominal antigen was important for the maintenance of long-term protective memory T cells (Gray and Matzinger, 1991; Oehen et al., 1992).
However, more recent studies have demonstrated that, per se, there is no need of the presence of the cognate antigen for T cells to survive; in the absence of nominal antigen, interaction of TCR with sp-MHC molecules is sufficient for the maintenance of $CD8^+$ memory T cells (Tanchot et al., 1997; Markiewicz et al., 1998) and $CD4^+$ T memory T cells (Garcia et al., 1999; Kassiotis et al., 2002). However, while in Markiewicz et al study, they showed that sp-MHC interaction is required for memory $CD8^+$ T cell maintenance because its survival was drastically impaired in

TAP1-deficient hosts; a more detailed study showed that $CD8^+$ memory T cells required only the non-restrictive class I interaction for survival (Tanchot et al., 1997).

As it was observed for naive T cells, conditional TCR ablation leads also to the disappearance of memory $CD8^+$ T cells (Polic et al., 2001), putting also in evidence the importance of TCR/sp-MHC for memory survival. However, other reports have demonstrated that memory $CD8^+$ (Murali-Krishna et al., 1999) and $CD4^+$ T cells (Swain et al., 1999) can persist respectively in β2m and I-Aβ deficient mice. Despite not being 100% MHC class I and MHC class II deficient mice, the MHC expression is strikingly reduced. The discrepancy in the results for the memory $CD8^+$ T might be due to the fact that the two first studies used as memory $CD8^+$ T cells, the HY cells, whereas in the later study, it was used P14 T cells or polyclonal $CD8^+$ T cells. It was shown that these two types of TCR Tg cells (aHY and P14) have different intrinsic properties, namely promiscuity (Hao et al., 2006), which plausibly explain the apparent contradictory results. As shown by Yi et al, promiscuous $CD8^+$ T cells can survive in a true MHC class I deficient mouse (H-2D/K/ß2m-knocked-out) by interacting with MHC class II molecules. In contrast to $CD8^+$ memory T cells, $CD4^+$ memory T cells yet persist after TCR ablation (Polic et al., 2001), suggesting that TCR tickling is not required for survival of most $CD4^+$ memory T cells. But despite memory $CD4^+$ T cells do not need p-MHC to survive, their function is impaired, for example, they lose the capacity to respond *in vivo* to antigen re-challenge or to provide help to B cell responses (Kassiotis et al., 2002). This indicates that functional memory (the parameter that really matters) needs some kind of periodic triggering by MHC.

The fact that it was shown that functional memory T cells need some kind of periodic triggering by MHC raises the question of whether cognate antigen causes optimal triggering. Therefore, one could think that the amount of nominal antigen present in the organism is a major factor for the maintenance or not of optimal memory T cell function. Nevertheless, LDP derived/memory-like T cells are continuously in the presence of antigens that activated them and, although they are not anergic or exhausted, it was shown that their fitness is lower compared to bona fide memory T cells (Bourgeois et al., 2005).

b) The role of cytokines

One cytokine that has emerged as a central regulator of the survival and/or turnover of memory T cells is IL-7. Its role was first unequivocally showed for the homeostasis of $CD8^+$ memory T cells (Schluns et al., 2000). The system that demonstrated this consisted on adoptive co-transfers of OT1 IL-$7R^{-/-}$ and normal OT1 (labeled with CFSE) cells into normal B6 hosts, which were then immediately immunized with VSV-OVA. Analysis of donor cell percentages was determined at different time points after immunization. It was observed that although both donor types had a similar division pattern; the survival of the $IL7R^{-/-}$ OT1 cells was impaired in the effector and especially in the memory phase.

Other papers also showed that IL-7 is in fact required for both polyclonal $CD4^+$ memory phenotype (Seddon et al., 2003) and TCR transgenic $CD4^+$ memory T cells (Kondrack et al., 2003; Li et al., 2003) survival. Nevertheless, $CD4^+$ memory T cells do not seem to be as susceptible to this cytokine as $CD8^+$ memory T cells, because it seems that TCR and IL-7R signals can have similar outcomes with respect to the maintenance of $CD4^+$ memory T cells, and in the absence of one factor, the other can counterbalance. In support of this is the fact that in the absence of both of these signals, $CD4^+$ memory T cells failed to survive (Seddon et al., 2003). In addition to the IL-7 requirement for memory $CD4^+$ T cell survival, the importance of this cytokine in the turnover of those cells has also been reported (Lenz et al., 2004).

Another important cytokine, but only for the homeostasis of memory $CD8^+$ T cells, is IL-15. The first evidence of its importance was the observation that it induced expansion of $CD8^+$ $CD44^{hi}$ T cells *in vitro* (Grabstein et al., 1994). Later on, the same function was demonstrated *in vivo* (Zhang et al., 1998). This specialized role was further put in evidence by the fact that over-expression of IL-15 leads to persistence of increased numbers of antigen-specific $CD8^+$ memory T cells after Listeria infection (Yajima et al., 2002) and IL-15 over-expressing transgenic animals contain increased numbers of memory phenotype $CD8^+$ T cells (Fehniger et al., 2001). Moreover, these findings were amplified by analysis of IL-$15^{-/-}$ and IL-$15R\alpha^{-/-}$ mice that displayed reduced numbers of $CD8^+$ T cells in the periphery, specially the subset of $CD8^+$ $CD44^{hi}$ T cells (memory phenotype) (Lodolce et al., 1998; Kennedy et al., 2000). The ability of IL-15 to reverse the $CD8^+$ T cell memory phenotype defect in otherwise naive IL-$15^{-/-}$ mice provides additional evidence to support a role for IL-15 in expansion and/or maintenance of these cells (Kennedy et al., 2000). However, it should be pointed out that these works were focused on analysis of memory

phenotype $CD8^+$ T cells resident in non-immunized mice, and thus the impact on antigen-specific $CD8^+$ memory T cells was not addressed. Nevertheless, two reports addressed this question, by studying $CD8^+$ antiviral responses to VSV (Schluns et al., 2002) or LCMV (Becker et al., 2002) infections in IL-$15^{-/-}$ and IL-$15R\alpha^{-/-}$ mice. Surprisingly, whereas IL-15 was required for primary expansion during VSV infection, this was not the case during LCMV infection. Nonetheless, both studies observed that IL-15 is required for the turnover of $CD8^+$ memory T cells to maintain populations of memory cells over long periods of time. Numbers of $CD8^+$ memory T cells undergo a slow attrition in the absence of IL-15. Another report implies that IL-15 is not only important in the turnover, but also in the survival of memory cells, because of a reduction in Bcl-2 expression in $CD8^+$ T cells deficient in the IL-15Rα chain (Wu et al., 2002b). However, the dependency of memory phenotype $CD8^+$ T cells can be overcome by overexpression of IL-7 (Kieper et al., 2002).

It should be just mentioned that although the majority of $CD8^+$ $CD44^{hi}$ T cells are dependent of IL-15 for their turnover/survival, there is a subset of $CD44^{hi}$ $CD8^+$ T cells that survive in IL-$15^{-/-}$ and IL-$15R\alpha^{-/-}$ mice and that are $CD122^{lo}$. This subset has a high rate of turnover (Judge et al., 2002).

In summary, like it was demonstrated for naive T cells, both memory T cell subsets ($CD4^+$ and $CD8^+$) need IL-7 for survival. The $CD8^+$ memory T cell maintenance is also governed by IL-15, especially for the $CD8^+$ memory T cell turnover.

3.2.4 Activation of memory T cells

a) Recall response to infections (conventional activation of memory T cells)

Like it was mentioned before, when rechallenged with an infectious pathogen, antigen-specific memory T cells, in contrast to naive T cells, can divide after a shorter lag time, have an increased division rate and show a more rapid and efficient differentiation to gain effector functions. It was also observed that, in contrary to naive cells, memory $CD8^+$ T cells are able to be activated by DCs in nonlymphoid tissues, although they are dependent on $CD4^+$ T cell help (Wakim et al., 2008). Furthermore, it was also shown that memory T cells can be stimulated by

all APCs, including B cells and macrophages to a limited extent (Croft et al., 1994); and it was postulated that reactivation of memory $CD8^+$ and $CD4^+$ T cells does not depend on CD28 co-stimulation (Croft et al., 1994; Bachmann et al., 1999b; Kim et al., 1999; London et al., 2000). However, a later report has shown that DCs are required for optimal secondary $CD8^+$ T cell responses (Zammit et al., 2005): the recall response to *Listeria monocytogenes*, to vesicular stomatitis virus (VSV) and to influenza virus was severely reduced (between 60 and 90% reduction) when DCs were depleted at the time of infection. This DC requirement suggests that memory $CD8^+$ T cell reactivation needs either co-stimulation and/or inflammatory cytokines derived from DCs. The requirement of CD28 co-stimulation for optimal memory $CD8^+$ T cell response was also demonstrated recently (Borowski et al., 2007).

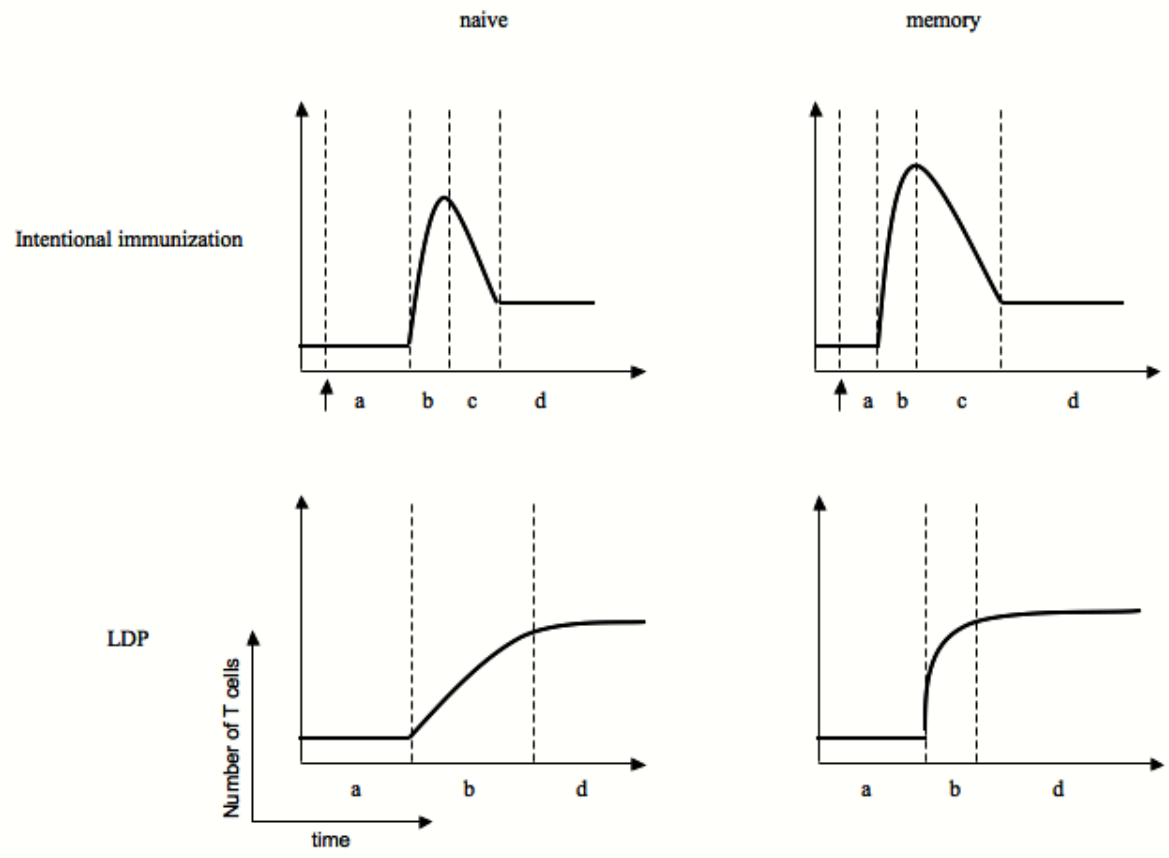
Altogether, despite the fact that memory T cells can be activated with less stringent requirements, for the best recall response, they need the signals that are indispensable for naive T cells, which are: TCR signaling through interaction with DCs, costimulation signaling, and possibly the presence of inflammatory cytokines (although there are no studies on this, at least to my knowledge).

b) LDP of memory T cells

As the survival and LDP of naive T cells seem to require the same kind of resources (p-MHC and IL-7); survival and LDP of memory T cells also share the same requirements. Therefore, like for memory T cell survival, IL-7 is important for LDP of both $CD8^+$ (Goldrath et al., 2002; Tan et al., 2002) and $CD4^+$ (Seddon et al., 2003) subsets, although $CD8^+$ memory T cells seem to be more dependent of this cytokine. Also, the $CD8^+$ subset needs the presence of IL-15 for LDP to occur (Becker et al., 2002).

Regarding p-MHC requirement, as it was demonstrated for memory T cell survival, LDP of memory T cells is less stringent to p-MHC than naive T cells. This conclusion comes from LDP comparing studies of naive and memory cells singularly transferred into lymphopenic hosts with or without deficiencies in MHC class (Murali-Krishna et al., 1999; Tanchot et al., 1997). The importance of TCR tickling with p-MHC was further confirmed by other studies. Another study documented the importance of p-MHC in $CD4^+$ memory T cells by showing that $CD4^+$ memory T cells with higher "avidity" (actually they should mention promiscuity) have a

competitive advantage over lower "avidity" T cells at early time points of LDP (Kassiotis et al., 2003). Altogether, we can conclude that TCR/p-MHC interactions are important in LDP of memory T cells, because in a competitive situation, cells interacting MHC and with higher promiscuity will out-compete the others.



legend: arrow means the moment of challenging

a) T cell activation

b) expansion and differentiation of T cells

c) contraction phase

d) steady-state of memory/memory-like T cells

Figure 5: **Differences in the kinetics of intentional immunization or LDP between naive and memory T cells**. This figure shows that in intentional immunization, naive T cell activation takes longer than memory T cells. For

LDP it is not really known if there is difference of time during initial activation, but it is known that memory T cells proliferate faster than naive T cells. One important difference between intentional immunization and LDP is that in LDP (although there is proliferation and cell death) the contraction phase (c) is absent.

3.3 T cell attrition

During the course of a lifetime, individuals are subjected not only to one infection, but to multiple pathogens, some homologous and others heterologous. Thus, the induction of profound T cell memory, particularly during viral infections poses a problem for the accommodation of memory T cells specific for multiple pathogens over the course of a lifetime. The memory T cell pool would rapidly fill up unless curtailing mechanisms operate. Although the numbers of $CD44^{hi}$ T cells increase with age, the size of the spleen and LNs restricts the total number of memory T cells.

An elegant work showed for the first time that sequential heterologous viral infections induce a reduction of otherwise stable virus-specific cytotoxic T memory cells (Selin et al., 1996). This phenomenon was later called attrition (Selin et al., 1999) and it was suggested that the mechanism that results in the attrition of memory T cells specific to viruses to which the host has been previously exposed may involve a selective process, as not all epitope-specific populations are reduced in equal proportions. Likewise, the immune system is able to maintain homeostasis by saving space with two mechanisms, either the deletion of a portion of the memory T cell pool or the preservation of the cross-reactive memory T cells. One report showed that attrition can also be observed in naive T cells, but the $CD44^{hi}$ T cells are the most affected population (McNally et al., 2001). This phenomenon was further extended to intracellular bacterial infections (Jiang et al., 2003b; Smith et al., 2002). However, while memory $CD4^+$ T cells seem resistant to depletion in a viral system (Varga et al., 2001), a report using a bacterial model showed that $CD4^+$ memory T cells are also susceptible to attrition (Smith et al., 2002). The reason for this differential susceptibility of $CD4^+$ memory T cells during viral vs bacterial attrition is not clear. One possibility is that since viral infections are overwhelmingly biased toward $CD8^+$ T cell responses, it is possible that attrition during such infections affects $CD8^+$ but not $CD4^+$ T cells. Another possible explanation may relate to the persistence of the pathogen

since the viral infection used for evaluating attrition did not cause chronic infections, whereas the bacterial infection that induced CD4+ T cell attrition was a chronic pathogen.

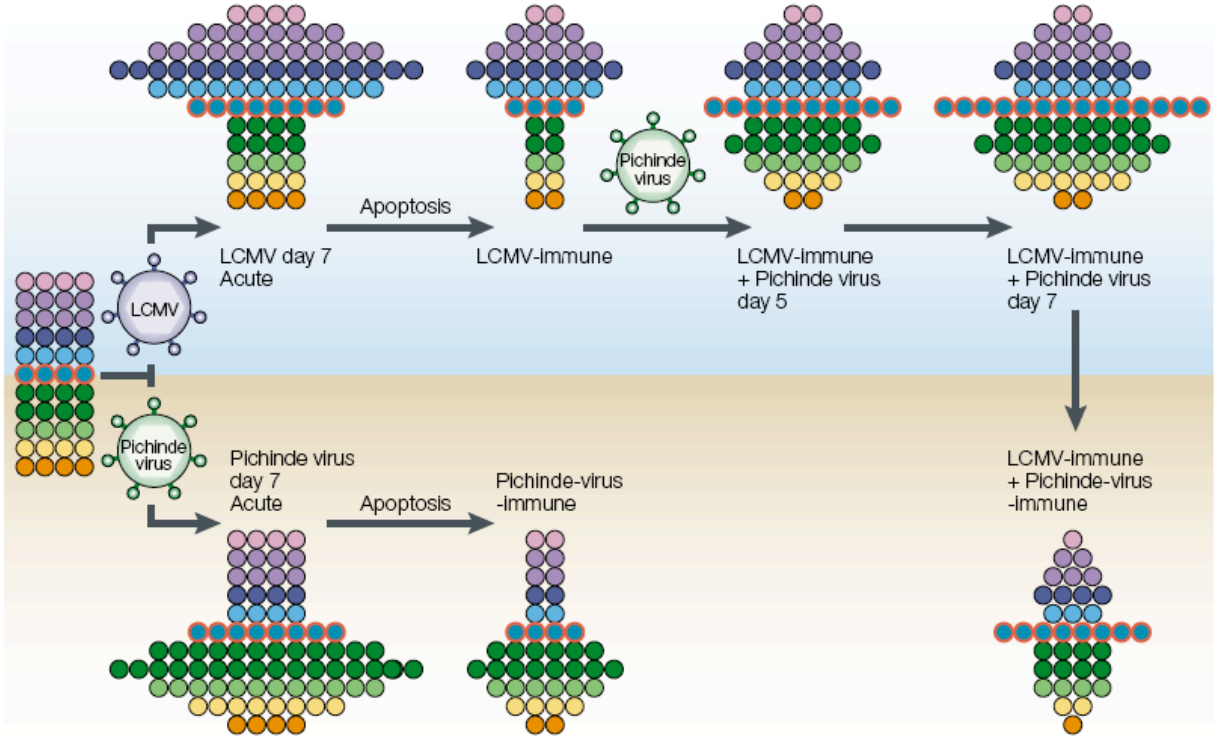


Figure 6: **Modulation of the T cell repertoire during viral infection**. A naive immune system (coloured dots represent T-cell populations with different specificities) is challenged with either of two heterologous viruses: LCMV or Pichinde virus. When the immune system that has been exposed to a virus (LCMV), is challenge by another virus (Pichinde virus), the cross-reactive T cells for the 2 viruses (red outline) will expand preferentially and dominate the response. The memory cells that were specific only for the first virus will be reduced in number. (From (Welsh and Selin, 2002).

3.3.1 Attrition models

Selin et al have proposed two hypothetical models to explain attrition :

-active deletion model suggests that pre-existing memory T cells undergo a bystander apoptosis and get killed off by cytotoxic factor(s) released during the early phase of a new infection.

- passive competition model suggests that during infection, pre-existing memory T cells inevitably have to compete with newly generated pathogen specific T cells for structural niches and/or survival factors such as cytokines, and eventually some memory T cells would lose this battle and die off.

A common feature of many severe viral (Oura et al., 1998; Summerfield et al., 1998; Reed et al., 2004) and bacterial (Hassan and Curtiss, 1994; Chapdelaine et al., 2003; Carrero et al., 2004) infections is a transient lymphopenia that occurs prior to the peak of the T cell response. Several reports demonstrated that depletion of memory T cells occurred very early (day 2) after viral (McNally et al., 2001; Kim and Welsh, 2004; Bahl et al., 2006) and bacterial (Jiang et al., 2003b) infections.

It was shown in one report that early memory $CD8^+$ T cell attrition occurred either in the presence or in the absence of cognate antigen, favoring therefore the active deletion model (Bahl et al., 2006). However, another study demonstrated a selective depletion of nonspecific memory T cells during the early phase of immune responses to infection, which more strongly supports the competition model (Jiang et al., 2003b). The reason for this discrepancy is not known. The difference between those two reports is based on the infection pathogen: in the first case a viral infection was used, while the second report used bacteria.

3.3.2 Attrition mechanism

It was observed that mice injected with poly(I:C) (which mimics double stranded RNA found in certain viruses) present a bystander T cell proliferation (Tough et al., 1996). Nevertheless, poly(I:C) induces a cell division pattern that does not result either in an increase in cell number or in changes in hierarchies (in contrast to what is found in heterologous viral infections) (Kim et al., 2002). However, during analysis of memory T cell numbers of poly(I:C)-treated mice in the early phase upon injection (between 12 and 72 hours), a decrease in memory

$CD8^+$ T cell numbers was observed (McNally et al., 2001; Jiang et al., 2005b; Bahl et al., 2006). Since it is known that poly(I:C) is an inducer of type I interferon through the TLR3 signaling pathway, it was hypothesized that a that a possible mechanism for attrition could involve IFNα and/or IFNβ. Interferons are natural proteins that belong to the large class of glycoproteins known as cytokines, and they are produced by a wide variety of cells in response to infections or tumors.

In some reports it was shown that the early decrease of lymphocytes was, indeed, due to type I interferon, and that decrease was the result of lymphocyte apoptosis. The observation came from the fact that type I IFN receptor deficient ($IFNAR^{-/-}$) mice displayed decreased lymphocyte apoptosis in both viral (McNally et al., 2001) and bacterial (Carrero et al., 2004; O'Connell et al., 2004) infections. However, other viral infection experiment demonstrated that the type I IFN induced initial decrease of lymphocytes upon infection was transient and it was not related to cell death, but rather to the regulation of lymphocyte recirculation and redistribution (Kamphuis et al., 2006). In this study they showed that type I IFNs had a direct role on T cells.

Altogether, it seems that depending on the infection, either we can have a real memory T cell attrition, with changes in the memory T cells hierarchies and frequencies, or just a trasient decrease that is later compensated and that keep T cell frequencies. In both types of early decreases, type I IFN seems to play a role but with different outcomes.

We cannot, however, exclude that it might exist other molecule(s) that can also have a role in the attrition and that probably their signaling pathways are somehow cross-linked with type I IFN. In support to this hypothesis is the fact that it was shown that IL-15, that is produced by APCs in response to IFNα/β and known for the maintenance and survival of memory $CD8^+$ T cells (Sprent and Surh, 2001), can also enhance the erosion of pre-existing memory $CD8^+$ T cells (Chapdelaine et al., 2003).

Altogether, the role of type I IFNs in attrition is not clear cut. More studies have to be performed to find whether type I IFNs play a general role in attrition or if it is restricted to some specific infections. Moreover, it is also of interest to determine if type I interferons have a direct role on T cells or if they play an indirect action. But we also have to keep in mind that other potential “attrition” molecules might exist.

In conclusion, if attrition has been first described as a mechanism affecting non-specific memory $CD8^+$ T cells during viral infections, the definitive nature of the target cells, $CD4^+$ versus $CD8^+$ T cells, memory versus naive T cells, specific versus non-specific cells, remains unclear and may depend on the infectious agents. Furthermore, in all cases, the mechanisms of this attrition process are still non elucidated.

Because until now the great majority of the studies only reported $CD8^+$ T cell attrition during infections inducing high $CD8^+$ T cell response, we decided to reconsider the possible attrition of $CD4^+$ T cells during a bacterial infection which induces a high $CD4^+$ T cell response, i.e. infection by Salmonella Thyphimurium.

4 SALMONELLA THYPHIMURIUM

The genus Salmonella is extremely heterogeneous, comprising almost 2000 serotypes, of which only a few are major human pathogens. However, despite this apparent complexity, Salmonella species are actually quite similar genetically, with the serotype differences based on surface antigen differences such as LPS and flagella.

Salmonella spp are gram-negative, flagellated and facultative intracellular pathogens capable of causing disease in a great variety of animal species, including human beings (Chalker and Blaser, 1988). Salmonella infections are largely initiated after the consumption of contaminated food or water (Chalker and Blaser, 1988). Ingested bacteria reach the intestinal tract where they interact in a rather intimate and complex manner with the cells that line the intestinal mucosa.

The etiologic agents of murine and human thyphoid fever are named Salmonella enterica serovar Thyphimurium and Salmonella enterica serovar Typhi. However, they are usually called Salmonella thyphimurium and Salmonella thyphi.

Host defense against Salmonella typhimurium infection requires significant contributions from both the innate and acquired arms of the immune system (Mittrucker and Kaufmann, 2000)

The initial stages of infection are characterized by an innate immune response triggered by pathogen associated molecular patterns (PAMPs) host recognition, such as flagellin, LPS,

lipoproteins and glycoproteins, which are recognized by Toll-like receptors (TLRs) and by nucleotide-binding oligomerization domains (NODs).

Experimental models using an injection of Salmonella have shown that neutrophils are rapidly recruited after bacterial administration (Coates and McColl, 2001; Fierer et al., 2002; Yang et al., 2002). Therefore, neutrophils have the earliest detectable change in abundance whereas changes in macrophages numbers are apparent a couple of days thereafter (Kirby et al., 2002). The number of DCs also increases within days after infection (Kirby et al., 2001). It has been demonstrated that Salmonella induces death of macrophages and DCs by activating the inflammasome (a caspase-1 activating multiprotein complex that processes pro-inflammatory cytokines including IL-1) (van der Velden et al., 2000). This kind of death is called pyroptosis, because in addition to cell lysis, there is release of pro-inflammatory cytokines that stimulate fever: IL-1β and IL-18 (Monack et al., 2001). These cytokines are important for the recruitment of immune cells, enhancement of T and NK cell function and production of diverse secondary cytokines (Braddock et al., 2004).

B6 background mice (the ones that we are going to use in our experiments) are Ity^s, which means that they have a point mutation in the Nramp1 that makes these mice susceptible among other infections, to Salmonella (Vidal et al., 1995). Therefore, macrophages from Ity^s mice cannot efficiently kill bacteria, and mice usually die before the adaptive response is initiated.

Although the innate mechanisms of the immune system are highly effective in restricting the initial growth of S. thyphimurium for several days, these mechanisms fail to achieve sterile elimination of the bacteria from the host. Only the generation of specific lymphocyte response allows the eventual effective eradication of bacteria, and provides increased protection against subsequent encounter with this pathogen.

T cell responses are important for primary and secondary immune responses: mice deficient in αβ T cells infected with attenuated strains fail to control infection and develop chronic disease (Hess et al., 1996; Sinha et al., 1997). In most Salmonella infection experiments, $CD4^+$ T cells were found to be more important than $CD8^+$ T cells. The depletion of $CD4^+$ T cells (as opposed to $CD8^+$ T cells) had a more pronounced effect on the control of primary Salmonella infection. Transfer of $CD4^+$ T cells from vaccinated mice into naive recipients resulted in higher levels of protection than transfer of $CD8^+$ T cells (Nauciel, 1990; Mastroeni et al., 1992; Sinha et al., 1997). Moreover, mice lacking MHC class II (deficient in $CD4^+$ T cells) succumb to infection

with strains of attenuated Salmonella that are normally eradicated in wild-type mice (Hess et al., 1996; Sinha et al., 1997). In contrast, mice lacking MHC class I (deficient in $CD8^+$ T cells), display no difference, or only a mild defect in the resolution of primary infection with attenuated Salmonella (Hess et al., 1996; Lo et al., 1999). This may explain the delayed expansion and contraction of $CD8^+$ T cells (Luu et al., 2006) and the massive proliferation of $CD4^+$ T cells (Srinivasan et al., 2004) observed in Salmonella infected mice, in sharp contrast to the vast majority of infections.

Because Salmonella thyphimurium is an intracellular facultative bacteria that induces, unusually, a much stronger $CD4^+$ T cell than $CD8^+$ T cell response, we decided to use this pathogen to study some of the open questions about attrition, including whether attrition can also occur in the $CD4^+$ T cell subset when the pathogen clearance is much more dependent on $CD4^+$ T cell responses and if IFNα/β plays an important role in Salmonella infection.

IN THIS THESIS:

As it was presented throughout the introduction, T cells are produced in the thymus (that belongs to what is called central compartment) and then migrate to the periphery. In the periphery there are two compartments that are considered to be independently regulated (Tanchot and Rocha, 1997): the naive and the activated/memory T cell compartments. Nevertheless, some resources required for T cells belonging to different peripheral compartments and ongoing different homeostatic processes are common, namely p-MHC and cytokines. Thus, in the first part of our study (see submitted article, p57), we decided to investigate if those common requirements could overlap between different T cell populations (naive/versus activated/memory cells) and between different homeostatic processes (survival and LDP), and to determine the impact of this potential overlapping on the establishment of the peripheral T cell pools.

In the second part of the study (see additional results, p81) we investigated attrition of memory T cells either in the absence or in the presence of bacterial infection, with the intention to understand if this phenomenon is selective of a specific T cell subset (CD8 versus CD4; naive versus memory…) and with the aim to discover what is the mechanism responsible for the deletion of T cells.

The results obtained will be discussed in the last chapter (Discussion, p100) of this thesis.

SUBMITTED ARTICLE

Classification:
Major category: Biological Science
Minor Category: Immunology

The role of TCR specificity and clonal competition during reconstruction of the peripheral T cell pool

Catarina Leitão[1], Antonio.A. Freitas[1] and Sylvie Garcia[1]

[1]Institut Pasteur, Lymphocyte Population Biology Unit, CNRS URA1961, F-75015 Paris, France

Correspondence to: Sylvie Garcia, PhD, Lymphocyte Population Biology Unit, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris cedex 15, France; phone: 33 1 45 68 82 56; fax: 33 1 45 68 89 21; e-mail address: sygarcia@pasteur.fr

Abbreviations: TCR, T cell receptor; sp-MHC, self-peptide-MHC; p-MHC, peptide-MHC; LDP, lymphopenia-driven proliferation; BM, bone marrow; Tg, transgenic; RAG, recombination activating gene; CFSE, carboxyfluorescein diacetate-succinimidyl ester; DN, double negative; DP, double positive; SP, single positive.

Abstract

Survival of peripheral $CD8^+$ T cells requires T cell receptor (TCR) interactions with peptide-MHC complexes (p-MHC). In the presence of homeostatic mechanisms that strictly control T cell numbers, it is likely that diverse T cells clones may compete for shared patterns of p-MHC. In the present study, we investigate if the recognition of p-MHC overlaps between different T cell populations and what role does this process plays in the establishment of the peripheral T cell pools. Using an experimental strategy that follows the fate of adoptively transferred polyclonal T cells into different types of RAG-deficient hosts, we demonstrate that T cells bearing different TCR specificities share identical TCR-specific requirements for survival and lymphopenia-driven-proliferation (LDP). This phenomenon of interclonal competition applies to both naive and activated/memory T cells and is partially determined by the clone size of the established/resident T cells. We also show that clonal competition with activated/memory resident T cells impacts differently on the fate of newly produced BM (bone marrow)-derived T cells or adoptively transferred peripheral T cells. Overall, our findings indicate that p-MHC are limiting resources, that define multiple diverse niches and can be shared by T cells belonging to different compartments. Moreover, they may have clinical relevance for disease-induced lymphopenia (HIV infection, irradiation, chemotherapy) potentially associated with T cell LDP and the T cell repertoire obtained after subsequent immune reconstitution.

Introduction

In the adult mouse, the number of peripheral T cells is maintained within narrow ranges and is largely independent of the numbers of precursor and thymus cells (1). Using competitive bone marrow (BM) reconstitution strategies to study the establishment of the peripheral $CD8^+$ T cell compartments, it was shown that, in the presence of continuous new cell production, each lymphocyte had to compete with other newly produced or resident cells for survival (2). Besides, competition is supposed to take place between T cells within a defined compartment, with naive and memory T cell pools regulated independently (3, 4)**.** Several different lines of evidence suggest the important role of TCR/self peptides-MHC complexes (sp-MHC) interactions in the process of T cell competition. First, TCR/sp-MHC interactions are required for peripheral T cell survival, as demonstrated by the findings that the absence of either the TCR (5, 6) or specific MHC molecules (7-11) leads to the disappearance of the peripheral MHC-restricted mature T cells. Secondly, in monoclonal (recombination activating gene) RAG-deficient TCR transgenic (Tg) mice, the number of T cells differs according to the TCR expressed (12), suggesting that TCR specificity for sp-MHC plays an important role to define the final clone size (12, 13). Finally, TCR recognition of sp-MHC has been shown to be required for the expansion of mature T cells during the peripheral T cell repopulation of immune-deficient hosts (1, 14-16). Alternatively, it was shown that MHC complexed with peptides derived from foreign Ags (p-MHC) could also participate to T cell LDP (17).

Because of the involvement of p-MHC complexes in many homeostatic T cell processes, competition among T cells in limiting conditions for defined TCR specific ligands are likely to occur. Different studies have showed that a defined TCR Tg T cell population transferred into hosts containing T cells bearing the same TCR Tg could not proliferate, in spite of the relative

host lymphopenia (19-22). These observations were consistent with the existence of competition between resident naive T cells and transferred T cells. Nevertheless, because most of these experiments involved T cells bearing identical TCRs, they did not allow any conclusion whether different T cell clones can compete for identical p-MHC. A recent study showed that lymphopenia-driven proliferation (LDP) of transferred polyclonal CD4 T cells was inversely correlated to the repertoire diversity of resident LDP-derived T cells (18). However, this study only addressed the role of the number and diversity of the resident T cell clones, and provided no information concerning the potential role of the TCR specificity of each of the competing T cell clones.

We have now studied the role of TCR specificity in T cell competition. Using different TCR transgenic RAG-deficient hosts, we show that diverse polyclonal $CD8^+$ T cell clones compete for identical p-MHC and that the clone size of resident T cells influenced the expansion and accumulation of the transferred T cells in a TCR-dependent manner. We show that this interclonal competition applied to both naive and activated/memory T cells. In addition, we studied clonal competition among naive and activated/memory T cells, showing that resident LDP-derived activated T cells interfere with the peripheral establishment of newly introduced populations of T cells. However, while newly produced BM-derived T cells were out competed according to their TCR specificity, the accumulation of a second population of transferred mature T cells occurred in a non-specific manner. These results indicate that p-MHC may define TCR-specific niches shared by T cell belonging to different compartments and controlled by different homeostatic processes.

Results

$CD8^+$ T cell LDP is regulated by interclonal competition.

In order to evaluate the influence of resident naive T cells and their TCR specificity on the fate of newly transferred T cells, we injected CFSE-labeled polyclonal B6 $CD8^+$ LN T cells into different lymphopenic $RAG^{0/0}$ hosts, non-transgenic (non-Tg) or transgenic (Tg) for different class I-restricted TCRs (aHY, P14 and OT1 Tg mice), i.e. hosts containing or not monoclonal $CD8^+$ T cell populations. As expected, after transfer into $RAG2^{0/0}$ hosts the transferred T cells expanded and the vast majority of cells recovered 7 weeks later had divided and lost their CFSE labeling (Fig. 1*A*). When the same number of T cells was transferred into TCR Tg $RAG2^{0/0}$ hosts, an increased fraction of the transferred T cells did not divide and remained $CFSE^{hi}$, following the order OT1>P14>aHY≥$RAG2^{0/0}$. Symmetrically, the number of $CFSE^-$ cells recovered in these hosts followed the opposite hierarchy $RAG2^{0/0}$>aHY>P14>OT1 (Fig. 1*B*).

Because the proliferation of some donor polyclonal $CD8^+$ T cells could be just delayed by the presence of the resident T cells, we evaluated the number of non-dividing ($CFSE^{hi}$) $CD8^+$ T cells after transfer into $RAG2^{0/0}$ or OT1 TCR Tg hosts at different time intervals. As shown in Figure 1*C*, the total number of $CD8^+$ T cells recovered from both $RAG2^{0/0}$ and OT1 hosts reached a plateau about 1 month after transfer. At equilibrium the number of T cells recovered in the OT1 hosts was 2.5 X lower than in the $RAG2^{0/0}$ hosts ($1.5*10^6$ vs. $3.5*10^6$). The accumulation of $CFSE^-$ T cells followed the same kinetics in both types of hosts (data not shown). In contrast, while in $RAG2^{0/0}$ hosts virtually all non-dividing ($CFSE^{hi}$) $CD8^+$ T cells disappear after transfer, in OT1 hosts some $CD8^+$ T cells still not divided even 2 months after

transfer (Fig. 1*D*). These findings indicate that the proliferation of a fraction of the polyclonal $CD8^+$ T cells was at least for 2 months stably prevented in OT1 hosts.

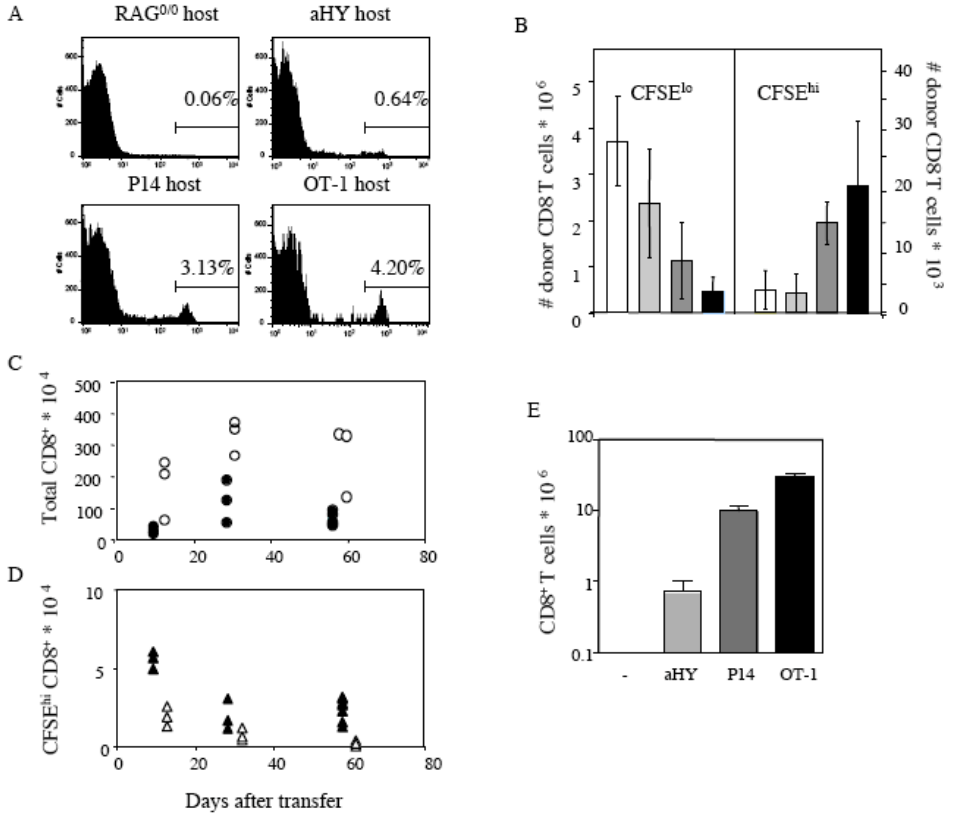


Fig. 1 : Proliferation of donor polyclonal $CD8^+$ T cells after transfer into different lymphopenic hosts. A) 2.10^6 CFSE-labeled $CD8^+$ $Thy1.1^+$ LN T cells were transferred into $Thy1.2^+$ $RAG^{0/0}$ hosts transgenic or not for the different class I-restricted TCRs: aHY, P14 and OT1. The CFSE profile of $CD3^+$ $CD8^+$ $Thy1.1^+$ is shown in each case. Each FACS profile is representative of 1 out of 5 mice for each type of hosts. Panel B) shows the numbers of donor $CFSE^{lo}$ (left part) and $CFSE^{hi}$ (right part) $CD8^+$ $Thy1.1^+$ LN T cells recovered in $RAG2^{0/0}$ (white bars), aHY (pale grey bars), P14 (dark grey bars) and OT1 (black bars) TCR Tg hosts. Each bar represents the mean ± SD of 5 mice. C) and D) show the total number (C) and the number of $CFSE^{hi}$ (D) donor $CD8^+$ T cells recovered at different times after the transfer of 2.10^6 polyclonal $Ly5.1^+$ $CD8^+$ LN T cells into $RAG^{0/0}$ (open symbols) or OT1 (closed symbols) hosts. E) shows the numbers of T cells in the different intact TCR Tg hosts.

We then asked about the mechanism that prevented division of a fraction of the transferred $CD8^+$ T cells in the TCR Tg hosts. This may be due to limitation of resources sequestered by the resident TCR Tg T cells to survive, and may be just related to their presence as T cells and

proportional to their respective number (Fig. 1*E*). More precisely, these limited resources may be either TCR-unspecific (IL-7, IL-15) or TCR-specific (likely p-MHC ligands) or both. We reasoned that if TCR-specific resources were involved in this inhibition, the proliferation of the undivided $CFSE^{hi}$ recovered from a defined TCR Tg host would be only specifically blocked by the presence of T cells from this specific host. To test this, we sorted polyclonal $CFSE^{-}$ and $CFSE^{hi}$ $Ly5.1^{+}$ $CD8^{+}$ T cells from primary injected OT1 hosts and transferred fewer than 10000 cells of each subset into different non-Tg and Tg Ly5.2 secondary $RAG^{0/0}$ hosts. The re-transferred $CFSE^{-}$ (re-labeled with CFSE) $CD8^{+}$ T cells divided extensively, losing their CFSE and becoming relatively easily detectable, whatever the type of secondary hosts they were transferred into (Fig. 2*A* and *C*). In contrast, the Ly5.1 $CD8^{+}$ $CFSE^{hi}$ T cells were not detectable in secondary OT1 $RAG2^{0/0}$ hosts, indicating their lack of proliferation after transfer into these hosts (Fig. 2*B*), but they divided extensively after transfer into non-Tg or P14 Tg $RAG2^{0/0}$ hosts (Fig. 2*B* and *C*). Thus, these observations exclude any intrinsic proliferation defect of these cells. They indicate that their inability to proliferate was dictated by the TCR specificity of OT1 T cells rather than due to competition for other non-specific factors like IL-7, IL-2 or IL-15 cytokines previously involved in $CD8^{+}$ T cell LDP (23, 24). Therefore, our findings confirmed that TCR-specific resources, likely recognition of p-MHC, control $CD8^{+}$ T cell LDP (23). These resources seem identical to those required by resident naive T cells to survive. They may define TCR-specific niches, which both control the number of $CD8^{+}$ T cells that survive and proliferate.

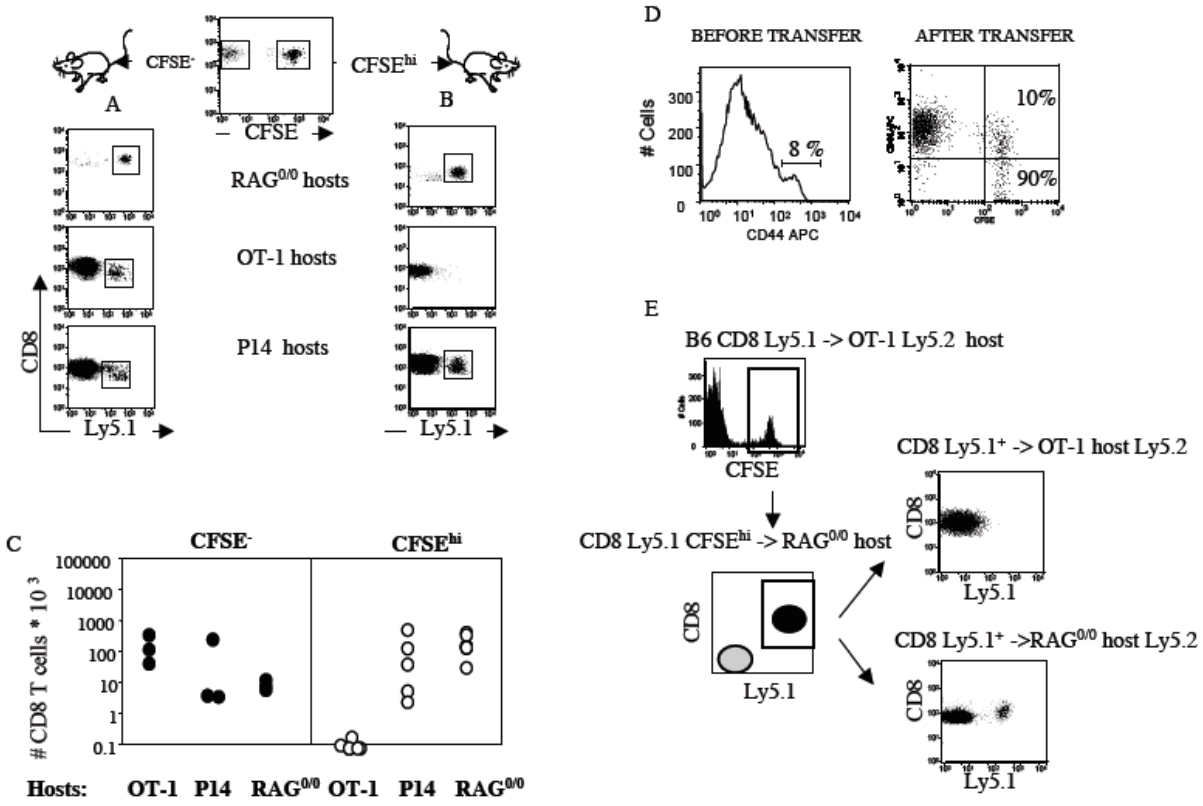


Fig. 2: Clonal inhibition of CFSE^{hi} donor CD8 T cells. Ly5.1$^+$ CD8$^+$ polyclonal T cells were injected into Ly5.2$^+$ OT1 RAG$^{0/0}$ hosts. Five to 6 weeks later, CFSE^{hi} and CFSE$^-$ Ly5.1$^+$ CD8$^+$ T cells were sorted and each population (after re-labeling with CFSE the CFSE$^-$ T cells) was re-transferred into new RAG$^{0/0}$, OT1 RAG$^{0/0}$ or P14 RAG$^{0/0}$ hosts. Six weeks later, the secondary hosts were sacrificed and the presence of donor Ly5.1$^+$CD3$^+$CD8$^+$ T cells derived from the CFSE$^-$ (A) and CFSE^{hi} (B) cell subsets was assessed by flow cytometry. C) shows the absolute number of CFSE^{hi} (white circles) or CFSE$^-$ (dark circles) derived CD3$^+$CD8$^+$Ly5.1$^+$ donor T cells recovered in the OT1 RAG$^{0/0}$, P14 RAG$^{0/0}$ or RAG$^{0/0}$ secondary hosts. This experiment is representative of 4 different experiments. D) Two million CFSE-labeled Ly5.1$^+$CD8$^+$ LN T cells were transferred into Ly5.2$^+$ OT1 RAG$^{0/0}$ hosts. The CD44 expression by the donor Ly5.1$^+$CD3$^+$CD8$^+$ T cells was assessed by flow cytometry before (histogram) and after transfer (dot plot) as a function of CFSE profile.

E) CFSE-labeled Ly5.1$^+$ CD8$^+$ polyclonal T cells were injected into Ly5.2$^+$ OT1 RAG$^{0/0}$ hosts. Six weeks later, CFSE^{hi} Ly5.1$^+$CD8$^+$ T cells were sorted and re-injected into secondary RAG$^{0/0}$ hosts. Six weeks later, the transferred Ly5.1$^+$CD8$^+$ T cells, which have expanded extensively (see Fig. 2B), were sorted again, re-labeled with CFSE and transferred into tertiary RAG$^{0/0}$ or OT1 RAG$^{0/0}$ hosts. Six weeks later, the expansion of the donor T cells in RAG$^{0/0}$ vs OT1 RAG$^{0/0}$ hosts was assessed by flow cytometry. Dot plots show CD8$^+$Ly5.1$^+$ donor T cells after gating on CD3$^+$ T cells. Please note that, while donor T cells were present in the tertiary RAG$^{0/0}$ hosts, we were unable to detect any in the tertiary OT1 RAG$^{0/0}$ hosts. This experiment is representative of 2 experiments.

Activated/memory T cells are also submitted to interclonal inhibition during LDP.

To determine whether naive and activated/memory $CD8^+$ T cells are equally affected by interclonal competition for TCR-specific ligands during LDP, we first characterized the phenotype of the $CFSE^{hi}$ $CD8^+$ T cells, which did not proliferate after transfer into TCR Tg hosts. We found that the representation of naive $CD44^{lo}$ (about 90%) and activated/memory $CD44^{hi}$ (about 10%) T cells among the transferred and the recovered non-dividing $CFSE^{hi}$ $CD8^+$ T cells was similar before and after transfer into OT1 hosts (Fig. 2D). This observation suggests that the LDP of both naive and activated/memory T cells was inhibited through TCR-specific interactions. More precisely, we studied whether the LDP of polyclonal activated/memory $CD8^+$ T cells was also inhibited by the presence of resident T cells. For this, we first transferred sorted $CFSE^{hi}$ polyclonal $CD8^+$ T cells, recovered from previous transfer into OT1 hosts, into RAG-deficient hosts. We tested whether these cells, once activated and expanded in these secondary RAG-deficient hosts, would be then able to expand in tertiary OT1 hosts. We found that, even after activation and extensive proliferation in secondary hosts, the $CD8^+$ memory-like T cells derived from previous $CFSE^{hi}$ T cells were still unable to proliferate and remained undetectable in new OT1 hosts (Fig. 2*E*). The same cells were able to proliferate and repopulate tertiary $RAG^{0/0}$ hosts (Fig. 2*E*). We concluded that resident host naive T cells inhibit, in a clonal specific manner, the LDP of not only naive but also activated/memory transferred $CD8^+$ T cells.

Clonal competition between different naive and activated T cell populations.

Since resident OT1 naive T cells could specifically prevent the proliferation of a fraction of polyclonal $CD8^+$ T cells, we asked whether these non-proliferating $CD8^+$ T cells could out compete the expansion of naive OT1 T cells during LDP. In other words, we wanted to study if the TCR-specific requirements of OT1 naive T cells and polyclonal $CD8^+$ T cells for survival and

LDP would somehow overlap. We co-transferred OT1 T cells together with polyclonal CFSE^{hi} CD8^{+} T cells, recovered from a primary transfer into OT1 hosts, into new RAG2$^{0/0}$ hosts. We found that 6 weeks after transfer the number of OT1 T cells recovered in the new hosts was significantly lower when co-transferred with CFSE^{hi} CD8^{+} T cells than when transferred alone, indicating that CFSE^{hi} CD8^{+} T cells could out-compete OT1 T cells (Fig. 3*A*). It should be pointed out that the co-transfer of OT1 cells with the CFSE^{-} CD8^{+} T cells recovered from primary OT1 hosts, did not modify the number of OT1 T cells recovered (Fig. 3*B*). To note, the number of CFSE^{-} CD8^{+} T cells recovered is reproducibly lower than that of CFSE^{hi} CD8^{+} T cells, likely because of a lower proliferative capacity due to their previous proliferation, which may be in part compensated by the help of OT1 T cells during co-transfer. Thus, the CFSE^{hi} CD8^{+} T cells, which did not divide in the first OT1 host mice, specifically out-competed the OT1 T cells during LDP, suggesting that the OT1 and the polyclonal CFSE^{+} CD8^{+} T cells recovered from OT1 hosts share similar TCR-specific resources to survive and proliferate.

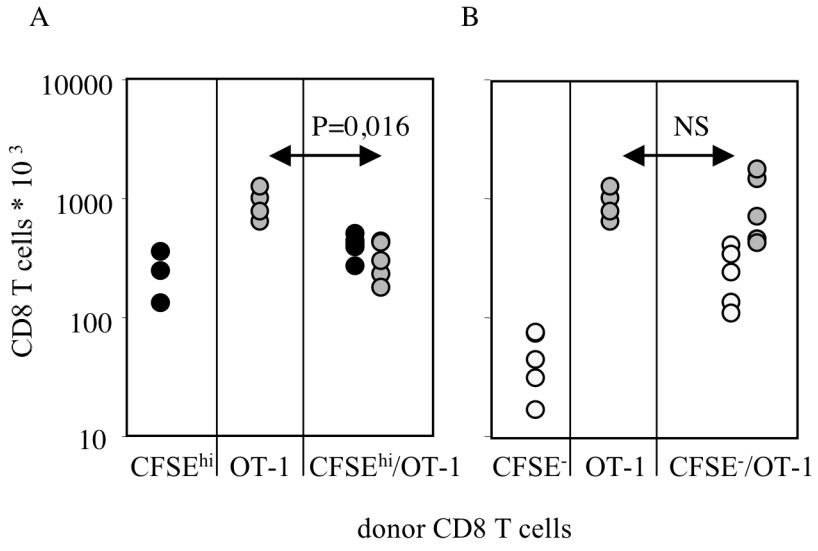


Fig. 3: Clonal competition during LDP between polyclonal CFSE^{hi} CD8 T cells and OT1 T cells. Ly5.1^{+} CD8^{+} polyclonal T cells were injected into Ly5.2^{+} OT1 RAG$^{0/0}$ hosts. Six weeks later, CFSE^{hi} (black circles, panel A) and

$CFSE^-$ (white circles, panel B) cells were sorted and injected alone or together with identical numbers of $Ly5.2^+$ OT1 T cells (grey circles in both A and B) into secondary $RAG^{0/0}$ hosts. Six weeks after transfer, the secondary $RAG2^{0/0}$ hosts were sacrificed and the absolute number of each population was calculated. P values (Mann-Whitney test) are indicated (NS = not significant). This experiment is representative of 3 experiments.

We next asked whether an established population of LDP-derived $CD8^+$ T cells could also affect the fate of newly introduced $CD8^+$ T cells, and whether these effects would be related to the TCR specificity of the populations involved. For this purpose, a first population of CFSE-labeled Ly5.1 TCR Tg T cells was transferred into $RAG^{0/0}$ hosts, followed 4 weeks later by a second population of Ly5.2 CFSE-labeled TCR Tg T cells. In control $RAG^{0/0}$ host mice each population was injected alone. Four weeks later, we studied both the CFSE dilution pattern and the number of cells recovered. The CFSE dilution profiles obtained showed that the presence of a first LDP-derived T cell subset reduced the proliferation of the second T cell subset and that this effect was more marked when resident T cells bore the same TCR as transferred T cells (Fig. 4*A-B*). However, although more marked when resident T cells were from the same specificity than transferred T cells, consistently with the CFSE profile, a decrease in the final number of the second injected population was observed with both homologous and heterologous resident T cells, indicating that the accumulation of the dividing T cells was inhibited partly in a non-TCR specific manner (Fig. 4*C-D*). It should be noted that the number of resident T cells was not statistically modified by the introduction of a new cell subset (data not shown).

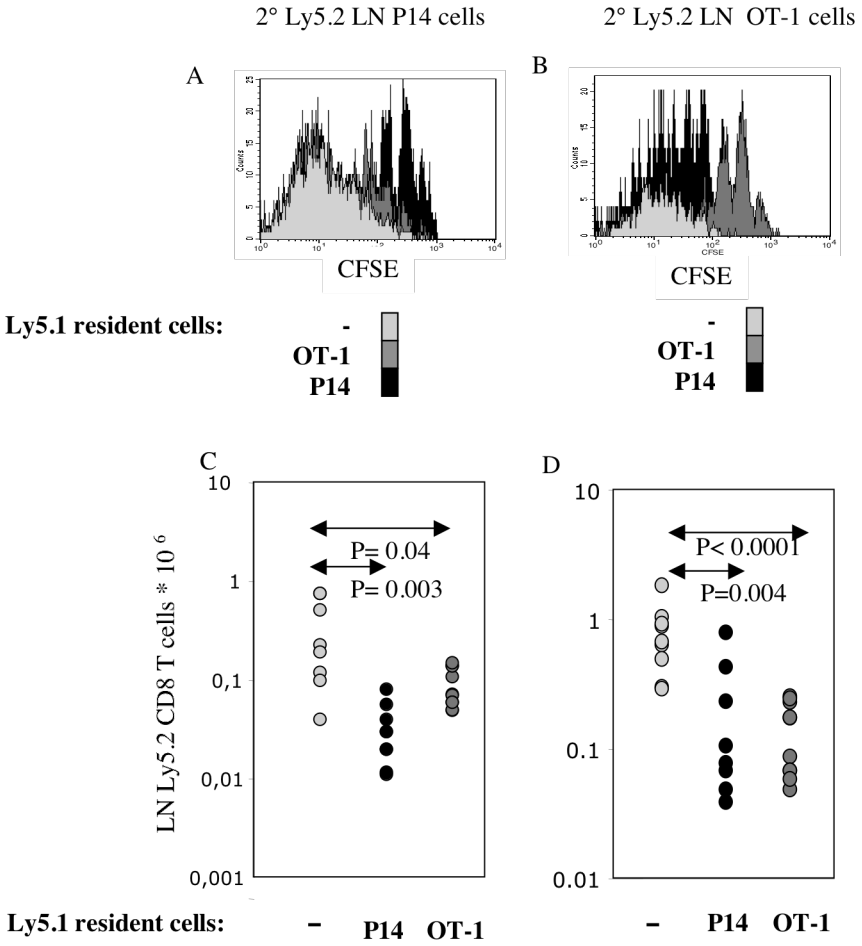


Fig. 4: Clonal inhibition of CD8 T cell LDP in hosts containing LDP-derived T cells. A first subset of Ly5.1 TCR Tg P14 or TCR Tg OT1 LN $CD8^+$ T cells was injected into $RAG2^{0/0}$ hosts. Four weeks later, a second subset of CFSE-labeled Ly5.2 TCR Tg P14 (A, C) or OT1 (B, D) LN $CD8^+$ T cells was injected into $RAG2^{0/0}$ hosts previously injected or not with a first subset of Ly5.1 TCR Tg P14 or OT1 LN $CD8^+$ T cells. Another 4 weeks later, the hosts were sacrificed and the CFSE profiles of the second subset of $Ly5.2^+CD3^+CD8^+$ P14 (A) or OT1 (B) cells were analyzed after transfer into new $RAG2^{0/0}$ hosts empty (pale grey histogram), or containing LDP-derived P14 (black histogram) or OT1 (dark grey histogram) T cells. Each histogram represents the data obtained from one mouse and is representative of 7-10 mice. C) and D) show the absolute number of cells of the second subset of Ly5.2 TCR Tg P14 (C) or OT1 (D) LN $CD8^+$ T cells recovered after transfer into $RAG2^{0/0}$ hosts empty (pale grey circles), or containing LDP-derived P14 (black circles) or OT1 (dark grey circles) T cells. P values (Mann-Whitney test) are indicated. The data shown are a pool of 2 different experiments.

We also studied whether the TCR specificity of a previously established population of LDP-derived $CD8^+$ T cells could affect the fate of a newly produced population of $CD8^+$ T cells.

For this, we first injected RAG2$^{0/0}$ hosts with TCR Tg LN T cells. Four weeks later, when the first population reached a stable plateau, we injected T cell-depleted BM cells from the same or different TCR Tg donors. Please note that the mice were not irradiated to preserve the existing first T cell population. In these conditions some of the transferred precursor cells colonize the empty RAG$^{0/0}$ thymus and reestablish T cell development. The mice were sacrificed 8 weeks later and the number of each of the T cells subsets evaluated. The presence or absence of the LDP-derived resident T cells did not alter the numbers of DN, DP and SP thymus subsets. Thus, resident T cells did not affect the thymus T cell development from the subsequently injected BM precursors (Fig. 5A) and the activation status of the BM-derived population in the periphery (Fig. 5B). However, as shown in Figure 5C, the number of BM-derived mature P14 and OT1 T cells was significantly decreased in the presence of LDP-derived T cells bearing the same TCR specificity, i.e. from the same TCR Tg donors. This was true for both naive CD44^{lo} and activated/memory CD44^{hi} T cells (data not shown), indicating that the peripheral seeding and establishment of newly incoming naive and activated/memory T cells is inhibited in a clonal specific manner by the presence of a previously established population of LDP-derived T cells. To note, the LDP-derived T cells were in all cases unaffected by the new incoming BM-derived T cells (data not shown). These results indicate that the peripheral accumulation of newly developing T cells is impaired by the presence of resident T cells bearing the same TCR specificity, i.e. in a TCR-dependent manner.

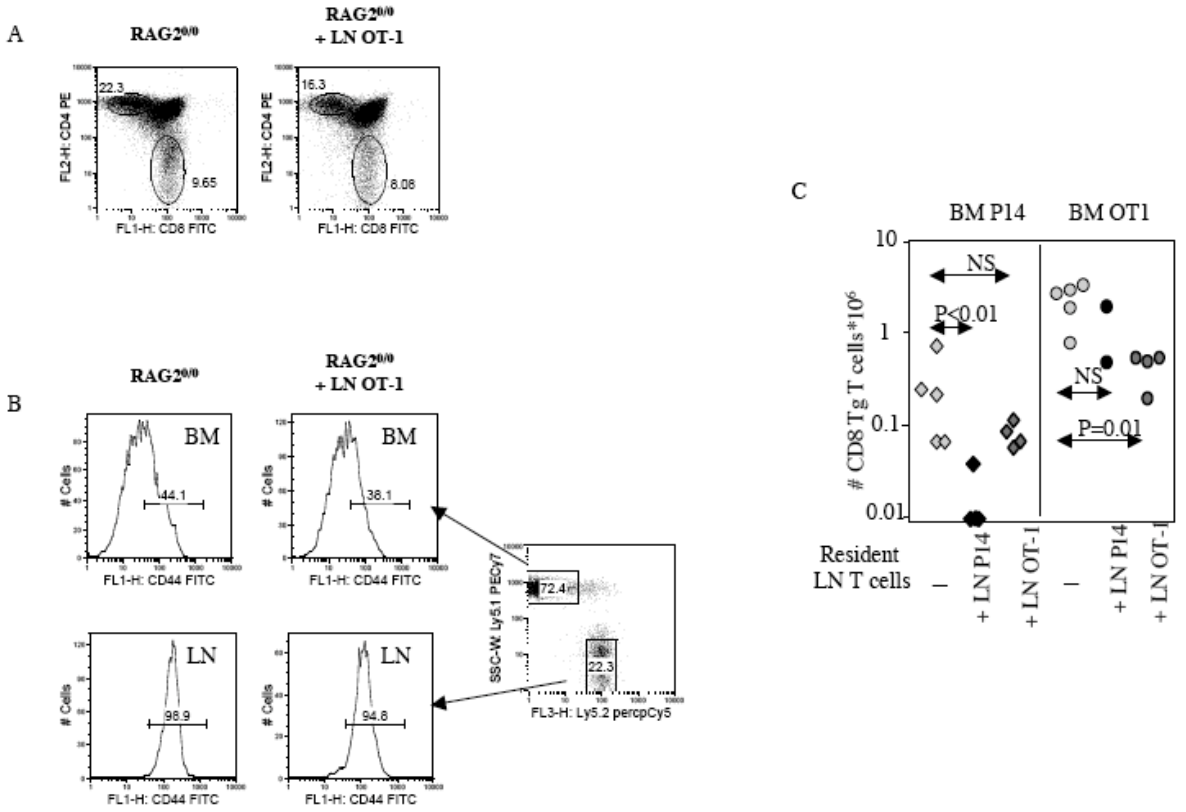


Fig. 5: Clonal inhibition of peripheral BM-derived T cell accumulation in hosts containing LDP-derived T cells. BM precursors from TCR Tg Ly5.1 P14 or OT1 mice were injected into empty RAG2$^{0/0}$ hosts or into RAG2$^{0/0}$ hosts previously injected 4 weeks earlier with LN TCR Tg Ly5.2 P14 or LN TCR Tg Ly5.2 OT1 CD8$^+$ T cells. Eight weeks later, the hosts were sacrificed. A) shows FACS profiles of CD4/CD8 thymocytes after reconstitution of RAG2$^{0/0}$ (left dot plot) or RAG2$^{0/0}$ hosts containing OT1 LN cells (right dot plot) by BM from OT1 donors. The percentages of SP CD4$^+$ and SP CD8$^+$ are indicated in each case. B) shows CD44 expression of peripheral BM-derived Ly5.1 OT1 T cells (dot plot and top histograms) and transferred LN Ly5.2 OT1 T cells (dot plot and bottom histograms) after transfer into RAG2$^{0/0}$ (left histograms) and RAG2$^{0/0}$ hosts containing OT1 LN cells (right histograms). C) the absolute numbers of BM-derived Ly5.1$^+$CD3$^+$CD8$^+$ were calculated for each types of donors and hosts. BM precursors from TCR Tg Ly5.1 P14 (left part) or OT1 (right part) mice were injected into empty RAG2$^{0/0}$ hosts (pale grey symbols) or into RAG2$^{0/0}$ hosts previously injected 4 weeks earlier with LN TCR Tg Ly5.2 P14 (black symbols) or LN TCR Tg OT1 (dark grey symbols) CD8$^+$ T cells. P values (Mann-Whitney test) are indicated. This experiment is representative of 3 experiments.

Discussion.

In the present study, we evaluated to which extend specific TCR/p-MHC interactions may overlap between different clones during $CD8^+$ T cell homeostasis. We studied the existence of interclonal competition between different subsets of $CD8^+$ T cells according to their TCR specificity, their differentiation state and the ongoing homeostatic processes. We found that clonal competition, based on specific TCR/p-MHC interactions and affecting both populations of naive and activated T cells, occurs during either peripheral cell accumulation after thymus development or peripheral T cell recovery after LDP.

It was previously shown that LDP of monoclonal TCR Tg $CD8^+$ T cells is fully blocked after transfer into hosts containing monoclonal T cells bearing the same TCR (19-22). We now extend this concept of "intraclonal competition" during LDP, to that of "interclonal competition" showing that the proliferation of some adoptively transferred polyclonal $CD8^+$ T cells was strictly and stably blocked by the presence of specific T cell clones in the lymphopenic hosts. This interpretation is strongly supported by the observation that the polyclonal $CD8^+$ T cells that were unable to proliferate in OT1 hosts specifically out-competed the OT1 T cells and that other T cell clones can not, when both T cell populations were co-transferred into $RAG2^{0/0}$ hosts. This indicates that these cells disclose the same TCR-specific ligands or requirements to survive and proliferate during LDP. In addition, we found that these requirements were shared by both naive and activated LDP-derived $CD8^+$ T cells. Although the sharing of p-MHC resources between surviving naive clonal and proliferating T cells suggests the involvement of MHC complexed with self peptide, we can not exclude any involvement of foreign peptides as there were previously implicated in T cell LDP (17).

The fraction of polyclonal $CD8^+$ T cells, whose proliferation was inhibited after adoptive transfer into TCR Tg hosts, varied according to the TCR specificity of monoclonal resident T

cells and followed the hierarchy OT1>P14>aHY hosts. It has been previously shown that the LDP of naive $CD4^+$ T cells and the number they reach, are not controlled by the number of resident T cells but by their repertoire diversity (18). In their study, Min et al. (18), generated RAG-deficient hosts containing identical numbers of memory $CD4^+$ T cells but with different T cell repertoire complexity by transfers of different numbers of naive $CD4^+$ T cells. They then showed that the expansion of a second polyclonal naive $CD4^+$ T cell population transferred into these different hosts was inversely proportional to the TCR diversity of the resident memory T cells (18). Since in the monoclonal RAG-deficient TCR Tg mice, the number of T cells varies according to the TCR expressed and was higher in OT1 than in P14 or aHY hosts, the differential expansion of polyclonal $CD8^+$ T cells that we observed in this study could be related in our case to the clone size of the resident T cells rather than to their diversity. Recent findings from our laboratory, showing that T cell clone size is determined according to the TCR promiscuity (12), reconcile our present results with the Min et al. report (18). Indeed, one can easily postulate that numerous and promiscuous TCR-bearing resident T cells, like OT1 T cells, will interact with many more p-MHC ligands than less promiscuous and numerous T cells, like aHY T cells. The number of T cell clones among the transferred polyclonal T cells that will not proliferate would be determined by the availability ofp-MHC ligands that are not sequestered by the resident T cells.

Using sequential transfers of mature T cells, we found that, while a population of resident naive cells selectively inhibited the proliferation and accumulation of newly transferred $CD8^+$ T cells bearing the same TCR specificity, LDP-derived resident T cells inhibited the expansion of all newly transferred T cells independently of their TCR specificity. They suggest that LDP-derived T cells may consume non-TCR specific resources that are required for the survival and accumulation of newly transferred T cells (22). IL-7 or IL-15 cytokines, which were shown to be

involved in memory T cell survival, are the most likely candidates. Alternatively, LDP-derived resident cells could actively inhibit or eliminate the newly proliferating T cells through TNFα or IFNα secretion, by a mechanism that was previously shown to be involved in attrition of memory $CD8^+$ T cells (25). Altogether, these observations strongly suggest that the survival requirements of naive and LDP-derived $CD8^+$ T cells do not completely overlap. Although they both require recognition of p-MHC, the activated LDP-derived T cells seemed to be more capable of using other "non-TCR specific" resources like cytokines.

By secondarily transferring BM precursors instead of mature T cells into hosts containing LDP-derived T cells, we found that the resident population selectively impaired the accumulation of newly produced recent thymus emigrants, in a TCR-specific manner. Most likely, the LDP-derived resident T cells monopolize TCR specific resources required by developing T cells to accumulate in the peripheral pools. In spite of the well-established different requirements of naive and memory T cells for survival (3, 4), it seems that they share enough resources to compete for them, at least in the case of the T cell clones we used and for TCR-specific resources.

In our TCR-specific system, resident activated clonal subsets were unaffected by the entering of new thymus-produced clonal T cells in the peripheral pools (data not shown). Although dealing with non-TCR specific issues, another study showed that polyclonal BM-derived T cells were able to out-compete $CD4^+$ TCR Tg LDP-derived T, while "true" memory A1 T cells were resistant to displacement (26). These later data likely reflect the high competitive capacities of polyclonal over TCR Tg T cells and/or different behaviour between $CD4^+$ and $CD8^+$ T cells. Thus, in normal conditions, new forming naive and/or memory T cells would replace the less competitive memory cells. This pre-emptive competition favors peripheral T cell diversity

since the new developing cells expressing redundant existing specificities do not survive. Interestingly, LDP-derived resident T cells affect differently recently developed T cells arising from BM precursors and adoptively transferred mature T cells, suggesting that these two latter populations have different survival requirement and/or competitive fitness. Alternatively, the kinetics and higher number of continuously produced RTE competitors, compared to the limited number of cells introduced upon adoptive transfer may justify the different final outcomes.

Overall, our results strongly support, that in the absence of intentional immunization, competition for overlapping p-MHC ligands controls peripheral $CD8^+$ T cell survival, proliferation and accumulation. This implies the existence of a complex network of TCR/p-MHC interactions defining specific niches, which regulate several homeostatic events and dictate the final composition of the T cell pools (27). The fact that resident naive T cells prevent the establishment of new T cells expressing identical TCR specificities, favors peripheral T cell repertoire diversity. On the other hand, the fact that LDP-derived activated T cells prevent, in a non-TCR specific manner, the accumulation of other activated T cell subsets, might enhance the efficiency of an immune response by avoiding bystander expansion of non-antigen specific cells and favoring the expansion of the appropriate set of responding cells. These findings may help understanding the homeostatic events leading to the establishment of a new T cell repertoire and occurring during the reconstitution of the peripheral T pools in individuals suffering from infection or therapeutic-induced lymphopenia, and potentially subjected to LDP processes.

Materials and methods

Mice. C57BL/6 (B6).Ly5.1, B6.PL.Thy1.1 and B6.RAG2$^{0/0}$ mice were from the Centre de Distribution, Typage & Archivage animal (CDTA, Orleans, France). The TCR Tg strains on a B6 background used in this study were: the aHY RAG2$^{0/0}$ mice Tg for a H-2D^{b}-restricted TCR specific for the HY male antigen; the P14 RAG2$^{0/0}$ mice Tg for a H-2D^{b}-restricted TCR specific for the gp33-41 epitope of the lymphocytic choriomeningitis virus glycoprotein (LCMV); the OT1 RAG1$^{0/0}$ mice Tg for a H-2K^{b}-restricted TCR specific for the 257-264 peptide of ovalbumin. All Tg mice were crossed to be Ly5.1^{+} and Ly5.2^{+} and were maintained in our SPF animal facilities according to the Experimental Ethics Committee guidelines.

Adoptive T cell transfers and CFSE staining. LN CD8^{+} T cells (2.10^{6}) from B6Ly5.1^{+} or B6.PL.Thy1.1^{+} mice (enriched after negative selection of CD19^{+} and CD4^{+} cells by magnetic cell sorting (MACS; Milteny Biotech)), were labeled with CFSE (Carboxy-Fluorescein Diacetate Succinimidyl Ester) (Molecular Probes) as previously adapted (28) and injected intravenously into RAG2$^{0/0}$ hosts, transgenic or not for different TCRs. For secondary T cell transfers, donor Ly5.1^{+}CD8^{+} CFSE^{hi} and CFSE^{-} T cells recovered from the first set of hosts, were sorted (MoFlow, DAKO) after depletion of TER119^{+}GR1^{+}Ly5.2^{+} cells using magnetic cell sorting (MACS; Milteny Biotech). The CFSE^{-} T cells were re-labeled with CFSE and about 3000-6000 cells from each subset were transferred into secondary RAG2$^{0/0}$, P14 RAG2$^{0/0}$ or OT1 RAG2$^{0/0}$ Ly5.2 hosts. In competition experiments, sorted CFSE^{hi} and CFSE^{-} (re-labeled with CFSE) donor CD8^{+} T cells were transferred alone or together with the same number of CFSE-labeled LN OT1 T cells into RAG2$^{0/0}$ hosts. Hosts were killed at different times after transfer. Spleen, inguinal and mesenteric LN cells were pooled and counted, and analyzed by flow cytometry. For the sequential cell transfer experiments, 2-3.10^{6} P14 or OT1 LN T cells were injected intravenously

into $RAG2^{0/0}$ hosts and 4 weeks later, a second subset of P14 or OT1 LN T cells ($2\text{-}3.10^6$) was injected into the same hosts. The mice were killed 4 weeks after the transfer of the 2nd population. In all the experiments, we used mice expressing different Ly5 and Thy1 allotypes to discriminate either T cells from hosts and donors or from different donors.

Flow cytometry. Pooled spleen, inguinal and mesenteric LN cells were stained with appropriate combinations of different FITC, PE, PercP or PercPCy5.5, APC, PE-Cy7 and APC-Cy7-conjugated CD4, CD8, CD3, Ly5.1, Ly5.2, Thy1.1, Thy1.2 and CD44 mAbs (BioSciences and Pharmingen). All acquisitions were done with LSR or Canto (Becton Dickinson) flow cytometers interfaced to the CellQuest or FlowJo softwares.

BM chimeras. $RAG2^{0/0}$ mice were injected i.v. with 2.10^6 LN T cells from P14 or OT1. Four weeks later, 5.10^6 T cell-depleted (by magnetic cell sorting, MACS; Milteny Biotech) BM precursors from P14 or OT1 donors were injected intravenously into the same hosts. Please note that the mice were not irradiated to preserve the existing first T cell population. In these conditions some of the transferred precursor cells colonize the empty $RAG^{0/0}$ thymus and reestablish T cell development. The mice were analyzed 8 weeks after the BM cell transfer. Donor and host T cells were discriminated on the basis of the differential expression of Ly5 alleles.

Acknowledgments: We thank Anne Louise from the Cytometry Platform at the Pasteur Institute for the FACS sorting experiments. CL is supported by a grant from the Portuguese Foundation for Science and Technology (FCT). This work was supported by the Pasteur Institute, the CNRS and grants from ANRS & ARC.

1. Almeida, A. R., Borghans, J. A., & Freitas, A. A. (2001) *The Journal of experimental medicine* **194,** 591-599.
2. Freitas, A. A., Agenes, F., & Coutinho, G. C. (1996) *European journal of immunology* **26,** 2640-2649.
3. Freitas, A. A. & Rocha, B. (1999) *Current opinion in immunology* **11,** 152-156.
4. Surh, C. D. & Sprent, J. (2005) *Seminars in immunology* **17,** 183-191.
5. Polic, B., Kunkel, D., Scheffold, A., & Rajewsky, K. (2001) *Proceedings of the National Academy of Sciences of the United States of America* **98,** 8744-8749.
6. Labrecque, N., Whitfield, L. S., Obst, R., Waltzinger, C., Benoist, C., & Mathis, D. (2001) *Immunity* **15,** 71-82.
7. Takeda, S., Rodewald, H. R., Arakawa, H., Bluethmann, H., & Shimizu, T. (1996) *Immunity* **5,** 217-228.
8. Tanchot, C., Lemonnier, F. A., Perarnau, B., Freitas, A. A., & Rocha, B. (1997) *Science (New York, N.Y* **276,** 2057-2062.
9. Brocker, T. (1997) *The Journal of experimental medicine* **186,** 1223-1232.
10. Kirberg, J., Berns, A., & von Boehmer, H. (1997) *The Journal of experimental medicine* **186,** 1269-1275.
11. Witherden, D., van Oers, N., Waltzinger, C., Weiss, A., Benoist, C., & Mathis, D. (2000) *The Journal of experimental medicine* **191,** 355-364.
12. Hao, Y., Legrand, N., & Freitas, A. A. (2006) *The Journal of experimental medicine* **203,** 1643-1649.
13. Kassiotis, G., Zamoyska, R., & Stockinger, B. (2003) *The Journal of experimental medicine* **197,** 1007-1016.
14. Jameson, S. C. (2002) *Nature reviews* **2,** 547-556.
15. Goldrath, A. W. & Bevan, M. J. (1999) *Immunity* **11,** 183-190.
16. Ernst, B., Lee, D. S., Chang, J. M., Sprent, J., & Surh, C. D. (1999) *Immunity* **11,** 173-181.
17. Kieper, W. C., Troy, A., Burghardt, J. T., Ramsey, C., Lee, J. Y., Jiang, H. Q., Dummer, W., Shen, H., Cebra, J. J., & Surh, C. D. (2005) *J Immunol* **174,** 3158-3163.
18. Min, B., Foucras, G., Meier-Schellersheim, M., & Paul, W. E. (2004) *Proceedings of the National Academy of Sciences of the United States of America* **101,** 3874-3879.
19. Kieper, W. C., Burghardt, J. T., & Surh, C. D. (2004) *J Immunol* **172,** 40-44.
20. Moses, C. T., Thorstenson, K. M., Jameson, S. C., & Khoruts, A. (2003) *Proceedings of the National Academy of Sciences of the United States of America* **100,** 1185-1190.
21. Troy, A. E. & Shen, H. (2003) *J Immunol* **170,** 672-676.
22. Ge, Q., Bai, A., Jones, B., Eisen, H. N., & Chen, J. (2004) *Proceedings of the National Academy of Sciences of the United States of America* **101,** 3041-3046.
23. Cho, J. H., Boyman, O., Kim, H. O., Hahm, B., Rubinstein, M. P., Ramsey, C., Kim, D. M., Surh, C. D., & Sprent, J. (2007) *The Journal of experimental medicine* **204,** 1787-1801.
24. Tan, J. T., Ernst, B., Kieper, W. C., LeRoy, E., Sprent, J., & Surh, C. D. (2002) *The Journal of experimental medicine* **195,** 1523-1532.
25. McNally, J. M., Zarozinski, C. C., Lin, M. Y., Brehm, M. A., Chen, H. D., & Welsh, R. M. (2001) *Journal of virology* **75,** 5965-5976.

26. Bourgeois, C., Kassiotis, G., and Stockinger, B. (2005). A major role for memory CD4 T cells in the control of lymphopenia-induced proliferation of naive CD4 T cells. J Immunol *174*, 5316-5323.
27. Freitas, A. A. & Rocha, B. (2000) *Annual review of immunology* **18,** 83-111.
28. Garcia, S., DiSanto, J., & Stockinger, B. (1999) *Immunity* **11,** 163-171.

ADDITIONAL RESULTS

1 MEMORY T CELL ATTRITION

The second part of our different study consisted in the analyzes of pre-existing memory T cell depletion in two situations ,i.e. in the absence of intentional immunization, or in the presence of a bacterial infection by Salmonella Thyphimurium.

1.1 IN THE ABSENCE OF INTENTIONAL IMMUNIZATION : « NATURAL ATTRITION »

We have previously shown that pre-existing memory-like T cells could interfere with the establishment of BM-derived T cells in a TCR-dependent manner. In these experiments, while the new incoming monoclonal T cells are affected by the presence of resident LDP-derived memory T cells bearing identical specificity, the number of resident memory-like T cells remain unchanged independently of the TCR of the BM-derived subset. However, this observation is in disagreement with two main studies. While reporting only a displacement of naive aHY $CD8^+$ T cells and not memory aHY $CD8^+$ T cells by BM-derived polyclonal T cells, the first study concluded an independent homeostatic regulation of naive and memory cells (Tanchot and Rocha, 1997). In contrast, the second study showed displacement of both clonal LDP-derived memory “like” and “true” (generated by immunization with the nominal antigen) memory TCR Tg A1 $CD4^+$ T cells by polyclonal BM-derived T cells (Bourgeois et al., 2005). The latter study reported however a preferential displacement of LDP-derived T cells, suggesting that LDP-derived memory-like T cells would be more susceptible to “natural attrition” by BM-derived T cells than “true” memory T cells. Because these discrepancies may arise from the use of different T cell specificities from $CD4^+$ (A1 TCR Tg) versus $CD8^+$ (aHY TCR Tg) lineages, and of LDP-derived versus “true” nominal antigen-induced memory T cells, we decided to reconsider the question of the depletion of pre-existing memory T cells using another experimental system.

We generated hosts containing BM precursors from different TCR Tg strains and containing clonal subsets of memory $CD4^+$ and $CD8^+$ T cells. We used the aHY and Marilyn TCR Tg strains to generate "true" memory T cells after activation by their cognate antigen (male antigen). In this study, all TCR Tg mice were on a $RAG^{-/-}$ background, ensuring the monoclonality of each subset.

The peripheral seeding of BM-derived T cells induces a reduction of resident memory T cell number.

One day after having injected a mixture of 10% BM from OT1 donors and 90% BM from OT2 donors into $RAG^{-/-}$ hosts, we co-injected aHY and Marilyn LN T cells together with male BMDCs. This last adoptive transfer led to the development of both $CD4^+$ Marilyn and $CD8^+$ aHY memory T cells. We previously established the 10% OT1 /90% OT2 BM ratio to avoid the thymic out-competition of OT2 thymic precursors by OT1 thymic precursors, due to the higher promiscuity of the OT1 TCR as compared to the OT2 TCR, leading to the general activation of the fewer OT2 recent thymic emigrants in the periphery (Freitas et al., 1996; Almeida et al., 2001). As control groups, we generated on the one hand chimeric mice with only BM-derived T cells; and on the other hand we generated chimeric mice constituted with only memory T cells. The identification of the different T cell populations was based either on the different Vα and Vβ expressions; or, in the case of OT1 and OT2 T cells that have commons Vα and Vβ chains, on the different congenic markers expression, like it is described in material and methods.

As shown in figure 1A, the CD44 expression of peripheral OT1 ($V\beta5^+$ $Ly5.1^+$) and OT2 ($V\beta5^+$ $Ly5.2^+$) T cells correspond to that observed in intact donors. Moreover, OT1 T cells are mainly $CD8^+$ $CD3^+$ (less than 2% $CD4^+$ $CD3^+$) and virtually all OT2 T cells are $CD4^+$ $CD3^+$ (data not shown). Figure 1B shows the activated/memory phenotype of aHY ($T3.70^+$ $CD8^+$ $CD3^+$) and Marilyn ($V\beta6^+$ $CD4^+$ $CD3^+$) T cells ($CD44^{hi}$) sixteen weeks after activation *in vivo* by male BMDCs. Functional tests also confirmed their capacity to secrete IFNγ upon short activation *in vitro* (data not shown), correlated with a faster response *in vivo* of the aHY T cell consistent with memory T cell generation in this adoptive transfer system (Schlecht et al., 2004).

The analysis of the hosts containing recently developed naive and resident memory T cell subsets showed that the seeding of BM-derived OT1 and OT2 T cells were not affected by the presence

of resident memory T cells (figure 1C). In contrast, the number of both $CD8^+$ aHY and $CD4^+$ Marilyn memory T cells were 6.5 times and 3.6 times respectively decreased in the presence of BM-derived OT1 and OT2 T cells (figure 1D). While the effect on $CD8^+$ memory T cells is reproducible (varying from 1.7 to 6.5 fold decrease), the effect on $CD4^+$ memory T cells varies from one experiment to another one. The generally relative lower number of $CD4^+$ memory T cells comparing to $CD8^+$ memory T cells, generated in this system, may explain the absence of a significant $CD4^+$ memory T cell deletion in some experiments. In addition, this displacement does not appear to be time-dependent (data not shown).

We named this displacement "natural attrition" as opposed to the attrition of bystander memory T cells observed during infections (see Introduction, chapter 3.3, p49).

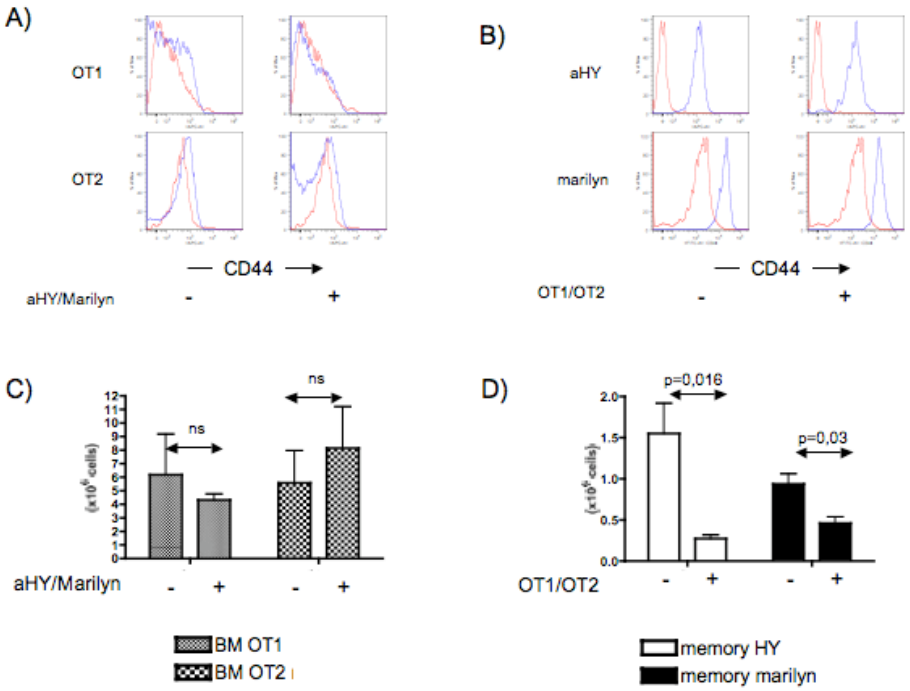


figure 1: Fate of BM-derived OT1 and OT2 T cells and memory aHY and Marilyn T cells

CD44 expression level (MFI) was analyzed by flow cytometry of both BM-derived OT1 (Vα2; Vβ5 Ly5.1) and OT2 (Vα2; Vβ5 Ly5.2) splenic T cells (A), and aHY (VαT3.70; Vβ8.2) and Marilyn (Vα1.1; Vβ6) memory splenic T cells (B). Red histograms correspond to the MFI of a naive T cell control, whereas blue histograms correspond to the cells found in the chimeric mouse. The numbers of BM-derived OT1 and OT2 spleen T cells (C) and aHY and Marilyn memory splenic T cells (D) were calculated in the presence (+) or absence (-) of the other two respective populations.

Depending on the TCR specificity, $CD8^+$ and $CD4^+$ BM-derived T cells are able, alone, to induce the attrition of $CD8^+$ memory T cells.

We next asked whether attrition of memory T cells was lineage specific. In other words, we wanted to test whether $CD8^+$ BM-derived T cells or $CD4^+$ BM-derived T cells alone were able to displace $CD8^+$ memory T cells (we focused on this subset, due to the low efficiency of attrition in the $CD4^+$ memory T cells, at least in our system). For this, we first assessed the fate of aHY memory T cells in the presence of OT1 BM-derived T cells. As shown in figure 2A, it appears that the introduction of a new population of thymus-derived OT1 T cells alone induced the attrition of aHY memory T cells, but did not affect Marilyn memory T cells. In contrast, the introduction of newly developed OT2 T cells alone had no effect on aHY memory T cells and on Marilyn T cells (figure 2B). We reasoned that this could be due to the lower number of peripheral OT2 T cells as compared to the OT1 T cells, linked to their lower TCR degeneracy (Hao et al., 2006), and not to their lineage. We, thus, performed the same type of experiment replacing BM precursors from OT2 donor strain by BM precursors from another class II-restricted TCR Tg donors. We choose the AND strain because of their well described TCR degeneracy (Kassiotis et al., 2003). As expected, we obtained much more AND T cells ($Vß3^+$ $CD3^+$ $CD4^+$) in the periphery of AND-BM engrafted hosts (figure 2C) than OT2 T cells after OT2 BM engraftment (figure 2B). Importantly, the vast majority of AND T cells were $CD4^+$ $CD3^+$ T cells cells (less than 5% were $CD8^+$ $CD3^+$) (data not shown). Figure 2C shows that BM-derived AND $CD4^+$ T cells alone were able to displace aHY $CD8^+$ memory T cells.

Altogether, these results suggest that the TCR degeneracy rather than the T cell lineage of newly introduced thymus-derived T cells is important for the induction of memory $CD8^+$ T cell attrition.

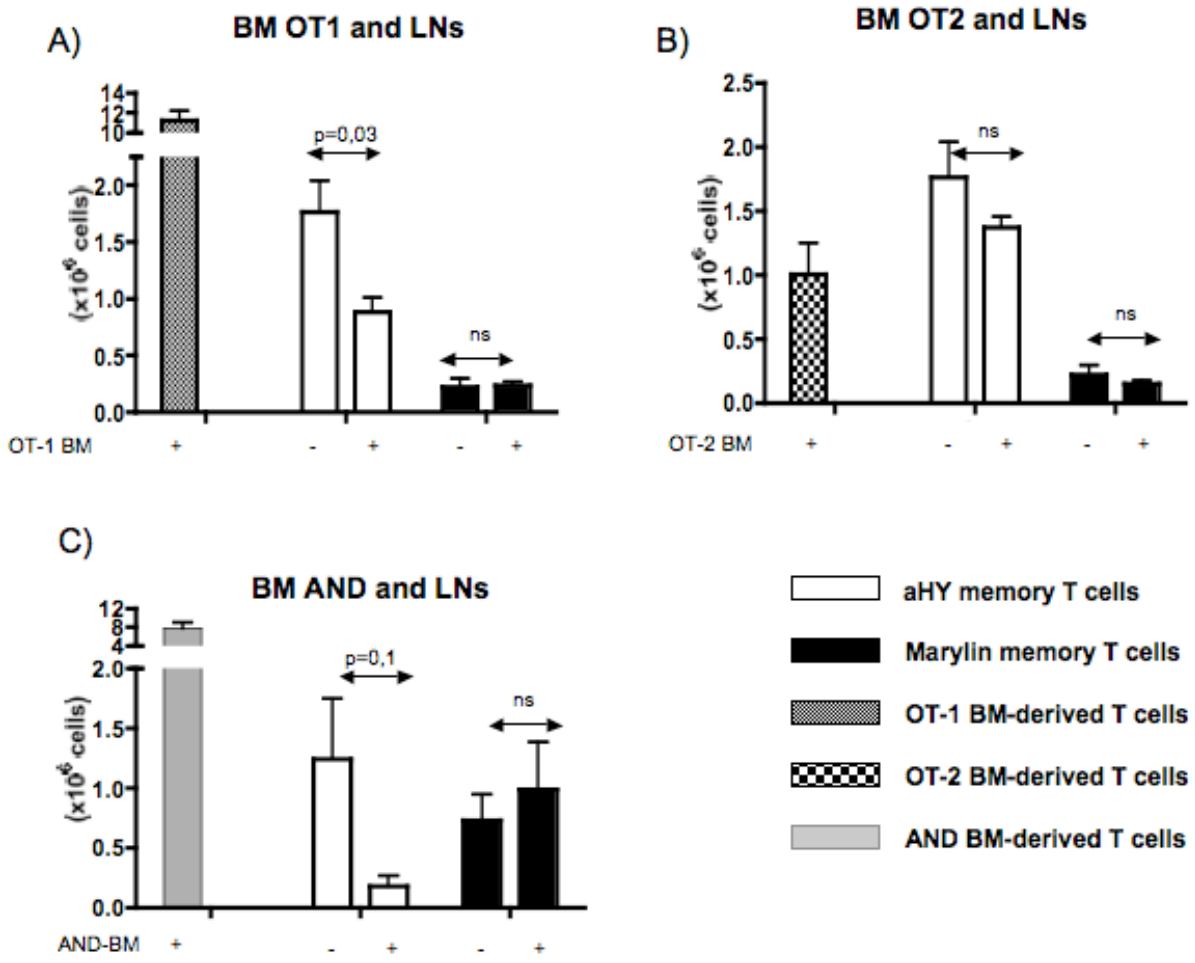


figure 2: Both BM-derived CD8$^+$ OT1 and CD4$^+$ AND TCR Tg T cells induce attrition of resident aHY memory T cells.

RAG2$^{-/-}$ recipients' females were constituted either with only memory aHY and Marilyn T cells or together with BM-derived OT1 T cells (A), BM-derived OT2 T cells (B) or BM-derived AND T cells (C). Between thirteen and fourteen weeks after transfer, total T cell numbers of pooled spleen and LNs (inguinal and mesenteric) were determined. Each bar represents the numbers in millions of BM-derived T cells and aHY and Marilyn memory T cells for each histogram.

"Natural attrition" does not affect either the phenotype or the function of remaining CD8$^+$ T cells.

In order to determine if the "natural" attrition process may influence the phenotype and function of the remaining memory aHY T cells, we compared the expression of some markers, including CD44, CD127 (the α chain of the IL-7R) and the CD122 (the ß chain of the IL-2R

shared by the IL-15R) of aHY memory T cells in the presence or absence of BM-derived T cells. We did not find any change in the expression of these markers (figure 3A), nor in the proportion of IFNγ-producing cells among both $CD8^+$ aHY memory T cells and $CD4^+$ Marilyn memory T cells (figure 3B).

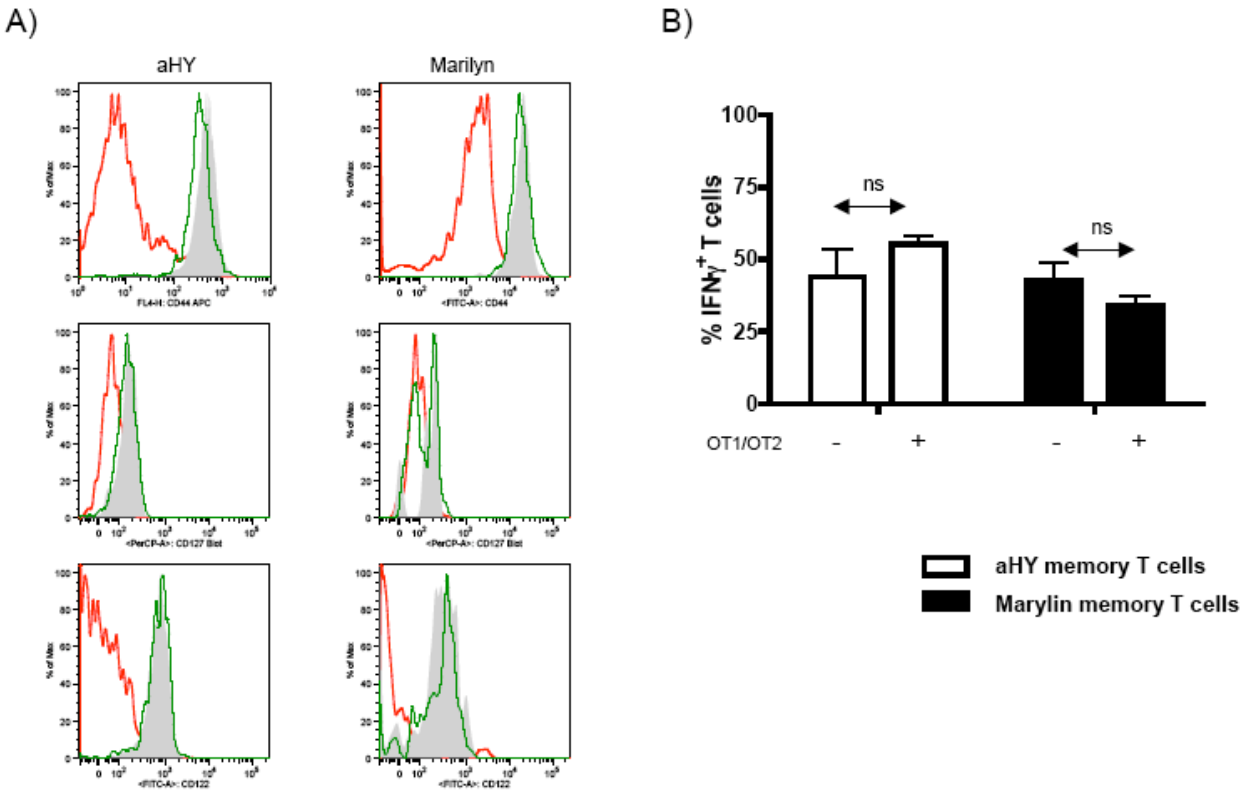


figure 3: phenotype and function of memory surviving aHY and Marilyn T cells

CD44, CD127 and CD122 expression profiles (expressed as MFI) were determined by flow cytometry for aHY and Marilyn memory T cells (A). The histogram delimited with a thick red line corresponds to the MFI of naive control TCR Tg mouse, the solid gray histogram corresponds to the MFI of memory TCR Tg T cells in chimeric mice with only memory T cell population, and the histogram delimited with a thin green line corresponds to the MFI of memory TCR Tg T cells in chimeric mice containing both memory and BM-derived OT1 and OT2 T cells. (B) The percentage of memory T cells producing IFNγ either in the absence (-) or in the presence (+) of BM-derived OT1 and OT2 T cells was also determined by flow cytometry after a short *in vitro* 4h-stimulation by PMA+ionomycin.

In conclusion, attrition of memory $CD8^+$ aHY T cells was always observed when TCR degeneracy of BM-derived T cells was high, independently of T cell lineage. For the $CD4^+$ Marilyn T cells, their attrition was not a constant factor. Therefore, although we observed that they can be displaced as well, they seem to be much more resistant to displacement.

1.2 DURING BACTERIAL INFECTION: "SALMONELLA-INDUCED ATTRITION":

As discussed previously in the introduction (chapter 3.3.1, p51), depletion of bystander non-specific memory T cells induced during viral and bacterial infections has been observed mainly for $CD8^+$ T cells, leading to the conclusion that $CD8^+$ T cells were predominantly susceptible to the attrition mechanism. $CD4^+$ memory T cell attrition has been only reported in the case of chronic *Mycobacterium bovis*. Since all tested infections mainly promote a $CD8^+$ T cell response, we decided to test the occurrence of $CD4^+$ and $CD8^+$ T cell attrition during infection by an attenuated strain of Salmonella thyphimurium, which induces a massive $CD4^+$ T cell response as previously described (Srinivasan et al., 2004). The reason of using an attenuated bacterium relies on the fact that we worked with mice on a B6 background. Those mice have a point mutation in the Nramp1 gene, located at the Ity/Bcg/Lsh locus on mouse chromosome 1 (Plant and Glynn, 1979), that makes B6 mice susceptible to Salmonella among other infections (Vidal et al., 1995). Indeed, macrophages from Ity^s B6 mice cannot efficiently kill bacteria.

To ensure the non-specificity of memory T cells, we used the system consisting on generating in $RAG^{-/-}$ hosts $CD8^+$ and $CD4^+$ memory T cells by adoptive transfer of Marilyn and aHY LN T cells together with BMDCs. We chose these TCR Tg strains because of the absence of degeneracy of their TCR, and the very weak probability to cross-react with Salmonella antigens. Host mice were then intraperitoneally infected with Salmonella (see Material and Methods, p97) and two weeks later, the numbers of aHY and Marilyn memory T cells were compared between non-infected and infected hosts. Each population was identified as described in Material and Methods.

Depletion in the spleen of both $CD8^+$ and $CD4^+$ memory T cells upon infection with Salmonella typhimurium.

Fourteen days upon intraperitoneal injection of either PBS (control group) or Salmonella, we analyzed in the host's spleen the total number of bacteria and the total number of memory $CD8^+$ aHY and $CD4^+$ Marilyn donor T cells. Mice infected with Salmonella present a splenomegaly, as a consequence of a huge increase of cells of the innate immune system, especially $CD11b^+$ macrophages (data not shown). Of note that intraperitoneal administration was used to mimic the systemic phase of the infection, bypassing the entry of the bacteria through epithelial cells in the gut.

The number of Salmonella presented in the spleen at day 14 upon infection was, in average, around $1x10^6$ (figure 4A). The Salmonella infection induced a reduction in cell number of both $CD8^+$ aHY (5 fold) and $CD4^+$ Marilyn (4 fold) memory T cells (figure 4B). On average, $CD8^+$ memory T cell decrease is higher than $CD4^+$ memory T cell decrease.

In conclusion, Salmonella seemed to be responsible not only for a decrease in memory $CD8^+$ T cell number, but also in memory $CD4^+$ T cell number.

A)

mouse	CFU/spleen
Not infected (control)	0
Infected nb1	$6,1x10^6$
Infected nb2	$2,1x10^5$
Infected nb3	$2,4x10^4$
Infected nb4	$1,0x10^5$
Infected nb5	$1,8x10^4$

B)

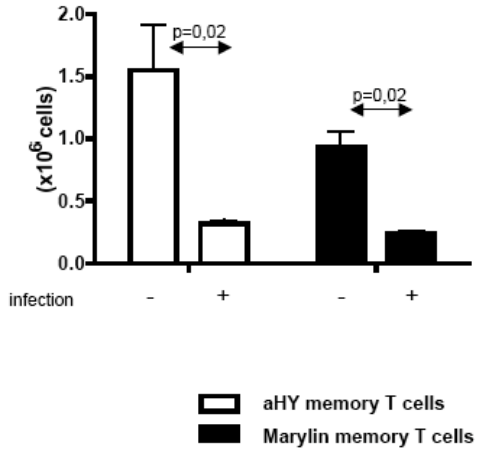


figure 4: Splenocytes and Salmonella colony counts

Female B6 $RAG2^{-/-}$ mice were adoptively transferred with aHY and Marilyn LNs cells along with male BMDCs. Fourteen weeks after adoptive transfer, mice were i.p. injected with $2,5x10^5$ Salmonella typhimurium or with PBS (control group). Fourteen days upon infection mice were sacrificed and spleen analysis was effectuated.

A) Determination of the number of colony forming units per spleen in the 5 infected mice. B) Recovered memory aHY (white columns) and Marilyn (black columns) T cell numbers in not infected (-) or infected (+) mice.

Depletion is related with apoptosis and not with T cell migration out of the spleen.

In order to determine whether this decrease in $CD4^+$ and $CD8^+$ memory T cell numbers was due to the depletion of these cells rather than to migration to other sites than spleen location, we also analyzed total donor $CD4^+$ and $CD8^+$ memory T cell numbers in the LNs (pooled inguinal and mesenteric), bone marrow (BM), liver and lung of infected versus non-infected "memory" hosts. As depicted in Table 1, total numbers of both $CD4^+$ and $CD8^+$ memory T cells decreased in infected mice, whatever the organ in question, although this diminish was more significant for $CD8^+$ aHY memory T cells. These results suggest that the decrease in T cell numbers is due to cell death instead of cell migration to other infected organs.

	BM		LNs (inguinal+mesenteric)		liver		lung		spleen	
	HY	marilyn	HY	marilyn	HY	marilyn	HY	marilyn	HY	marilyn
Not infected	0,26* +/- 0,13	0,01 +/- 0,01	3,68 +/- 1,07	0,42 +/- 0,18	0,24 +/- 0,09	0,02 +/- 0,01	2,46 +/- 0,38	0,37 +/- 0,23	1,94 +/- 0,33	0,26 +/- 0,07
infected	0,05 +/- 0,05	0	0,87 +/- 0,6	0,17 +/- 0,05	0,02 +/- 0,01	0	0,31 +/- 0,15	0,15 +/- 0,07	0,53 +/- 0,4	0,14 +/- 0,08

* Note: CD4+ CD3+ Vß6+ and CD8+ CD3+ T3.70+ T cell numbers X 10^6

Table 1: memory T cell numbers in different organs

Numbers of aHY and Marilyn T cells were determined in infected and not infected chimeric mice. The following organs were analyzed 14 days upon infection: bone marrow (BM), LNs (pooled inguinal and mesenteric), liver and lung. Values in the table are in million ($x10^6$) and represent the mean+/- standard deviation of 5 mice.

To further document this hypothesis, we compared the percentage of apoptotic T cells, detected by 7AAD incorporation (when cell starts losing the impermeability, 7AAD as being a

small molecule can pass through the cell membrane and intercalate the DNA), and the percentage of T cells expressing the anti-apoptotic molecule Bcl-2 in infected and non-infected mice. We observed that the percentage of $7AAD^+$ T cells was higher in infected mice than non-infected (figure 5A). The increase of apoptotic T cells was more pronounced in the $CD8^+$ memory T cell subset than in the $CD4^+$ memory T (figure 5A) .

We also checked if the expression of the anti-apoptotic molecule Bcl-2 was altered in infected mice. We observed a decrease in the percentage of memory T cells expressing Bcl-2 in infected mice compared to non-infected mice (figure 5B), although this reduction was much stronger for the $CD8^+$ aHY memory T cells than for the $CD4^+$ Marilyn memory T cells (figure 5B).

Altogether, these results indicate that the decrease of both $CD4^+$ and $CD8^+$ T cells during Salmonella infection are due to their death. They also suggest that this death may be regulated by Bcl-2.

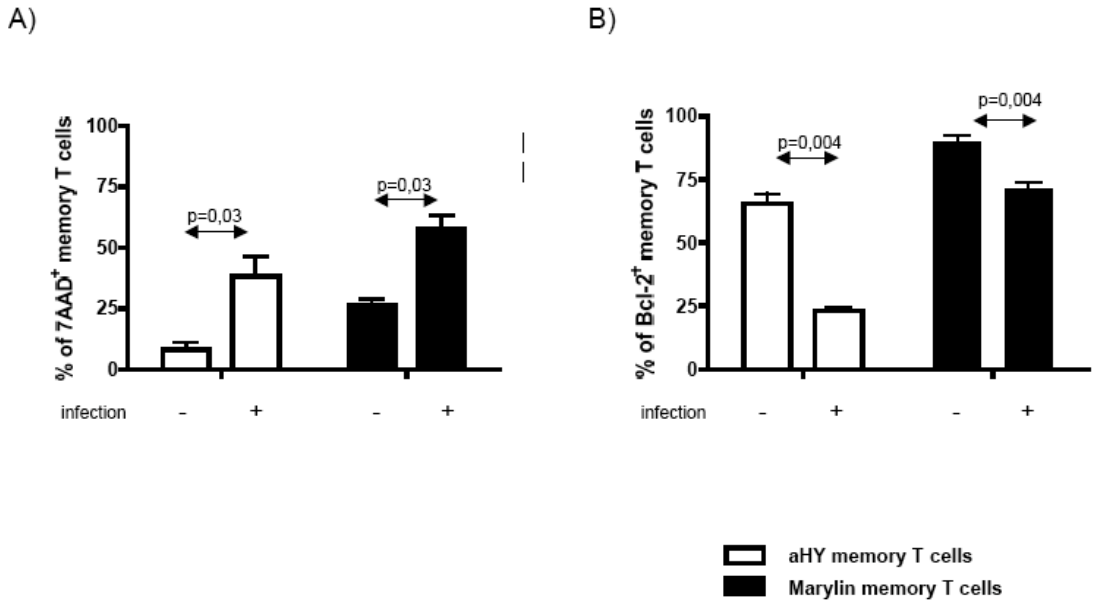


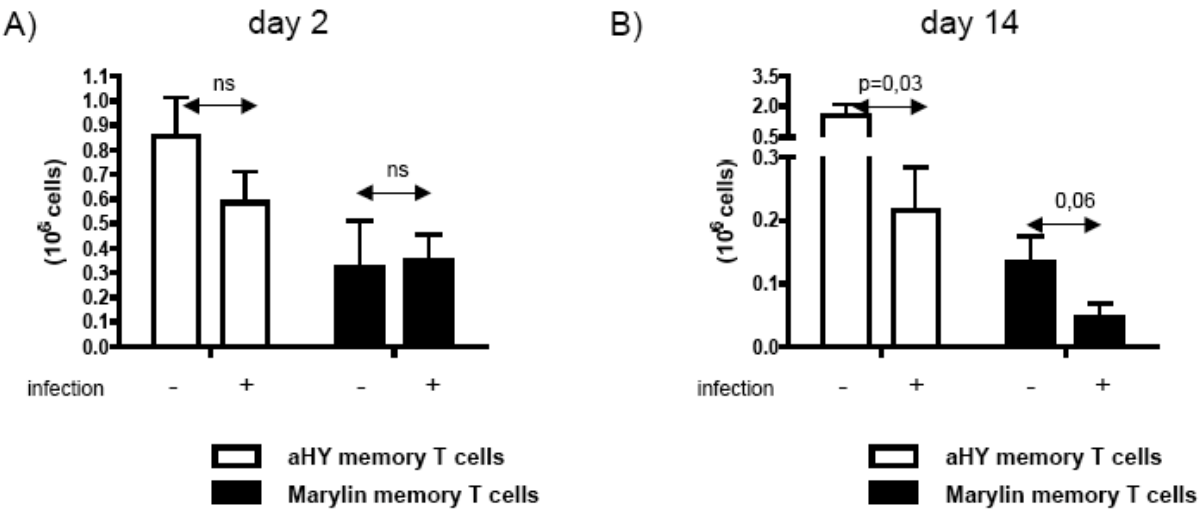
figure 5: Study of the fragility state of memory T cells in infected and non-infected mice

Six weeks after i.v. injection of LN aHY and Marilyn cells plus male BMDCs into B6 $RAG2^{-/-}$ female recipients, mice were infected with Salmonella typhimurium, and 14 days later, mice were killed and splenocytes were analyzed. Control mice were i.p. injected with PBS.

Determination of the percentage of apoptotic ($7AAD^+$) A) and high Bcl-2 expression level B) in memory aHY (white bars) and Marilyn (black bars) T cells in infected (+) and non-infected (-) mice.

Memory attrition is not early induced during Salmonella infection: it is related to the bacterial load

To understand the mechanism responsible for this Salmonella-induced depletion of non-specific $CD4^+$ and $CD8^+$ memory T cells, we first asked whether this process could occur in the very early phases of infection, as it was previously described for other infections (McNally et al., 2001; Jiang et al., 2003b; Kim and Welsh, 2004; Bahl et al., 2006). For this, we compared the fate of aHY and Marilyn memory T cell subsets 2 and 14 days after infection by Salmonella. At day 2, we did not find any alteration in the number of memory $CD8^+$ aHY and memory $CD4^+$ Marilyn T cells (figure 6A), whereas at day 14 attrition was observed for both memory T cell subsets, with again a higher effect on $CD8^+$ than $CD4^+$ T cells (figure 6B). Consistently, while we did not observe any difference in the percentage of apoptotic cells in infected and non-infected mice at day 2, we found an increase in the percentage of $7AAD^+$ cells in the infected group at day 14 (data not shown). When we looked at the Salmonella colony forming units (CFU)/spleen, at day 2 either bacteria were not detectable or the number of CFU/spleen was very small, between 50 and 100 (figure 6C). In contrast, at day 14, the CFU/spleen was between $1,4x10^3$ and $1,7x10^4$ (figure 6D). These results suggest that the depletion of memory T cells does not occur at early time point of infection and, that it is correlated with the bacterial load.



C)

mouse	CFU/spleen
Not infected (control)	0
Infected nb1	0
Infected nb2	50
Infected nb3	104
Infected nb4	0

D)

mouse	CFU/spleen
Not infected (control)	0
Infected nb1	$3,1x10^3$
Infected nb2	$8,5x10^3$
Infected nb3	$1,7x10^4$
Infected nb4	$1,4x10^3$

figure 6: Kinetics of Salmonella typhimurium infection

Six weeks after i.v. LNs aHY and Marilyn and male BMDCs co-transfer into B6 $RAG2^{-/-}$ female recipients, mice were infected with Salmonella typhimurium. Mice were then sacrificed at day 2 and day 14 post-infection.

Numbers of aHY (white bars) and Marilyn (black bars) T cells in infected (+) and not infected (-) mice obtained from the spleen at day 2 A) and day 14 B) upon i.p. infection.

C) and D) is the CFU counts per spleen in the different infected mice 2 and 14 days after infection, respectively.

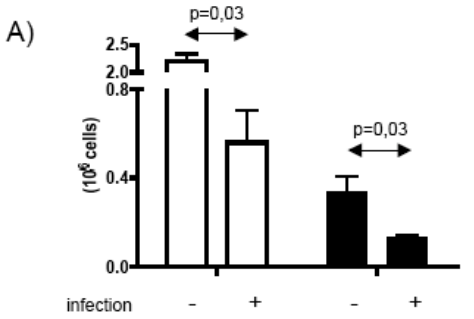
Memory attrition is not directly induced by type I IFN

Because former reports have attributed a role of IFNα/β in the attrition induction of memory $CD8^+$ T cells (McNally et al., 2001; Carrero et al., 2004), we asked whether type I IFNs have a direct role on memory T cells attrition. For this, we generated $IFNAR^{-/-}$ $CD8^+$ aHY and $CD4^+$ Marilyn memory T cells and compared their fate with wild type memory T cell subsets. $IFNAR^{-/-}$ mice lack both IFNAR1 and IFNAR2 chains that form the IFNα/β receptor; therefore no type IFN-mediated signaling can be transmitted to $IFNAR^{-/-}$ cells. We first ensured that $CD4^+$

and $CD8^+$ $IFNAR^{-/-}$ T cells have no functional abnormalities and no problems in migration and LDP (data not shown), confirming previous observations (Muller et al., 1994).

Spleen analysis 14 days upon infection showed that both wild type memory T cells (data not shown) and $IFNAR^{-/-}$ memory T cells (figure 7A) decreased in numbers in a similar extent. The CFU/spleen was high and also similar for both wild type (data not shown) and $IFNAR^{-/-}$ memory T cell (figure 7B) groups. As seen for wild type memory T cells (figure 5B), $IFNAR^{-/-}$ memory T cells also present a reduction, stronger for the $CD8^+$ subset than for the $CD4^+$ subset, of the percentage of cells expressing high levels of Bcl-2 (figure 7C).

Altogether, these results suggest that type I IFN-mediated signals were not directly responsible for the depletion of both $CD4^+$ and $CD8^+$ non-specific T cell memory during Salmonella infection.



B)

mouse	CFU/spleen
Not infected (control)	0
Infected nb1	$2,8x10^5$
Infected nb2	$1,3x10^5$
Infected nb3	$9,3x10^4$
Infected nb4	$1,2x10^5$

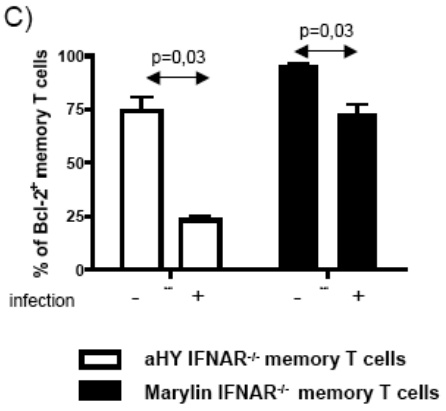


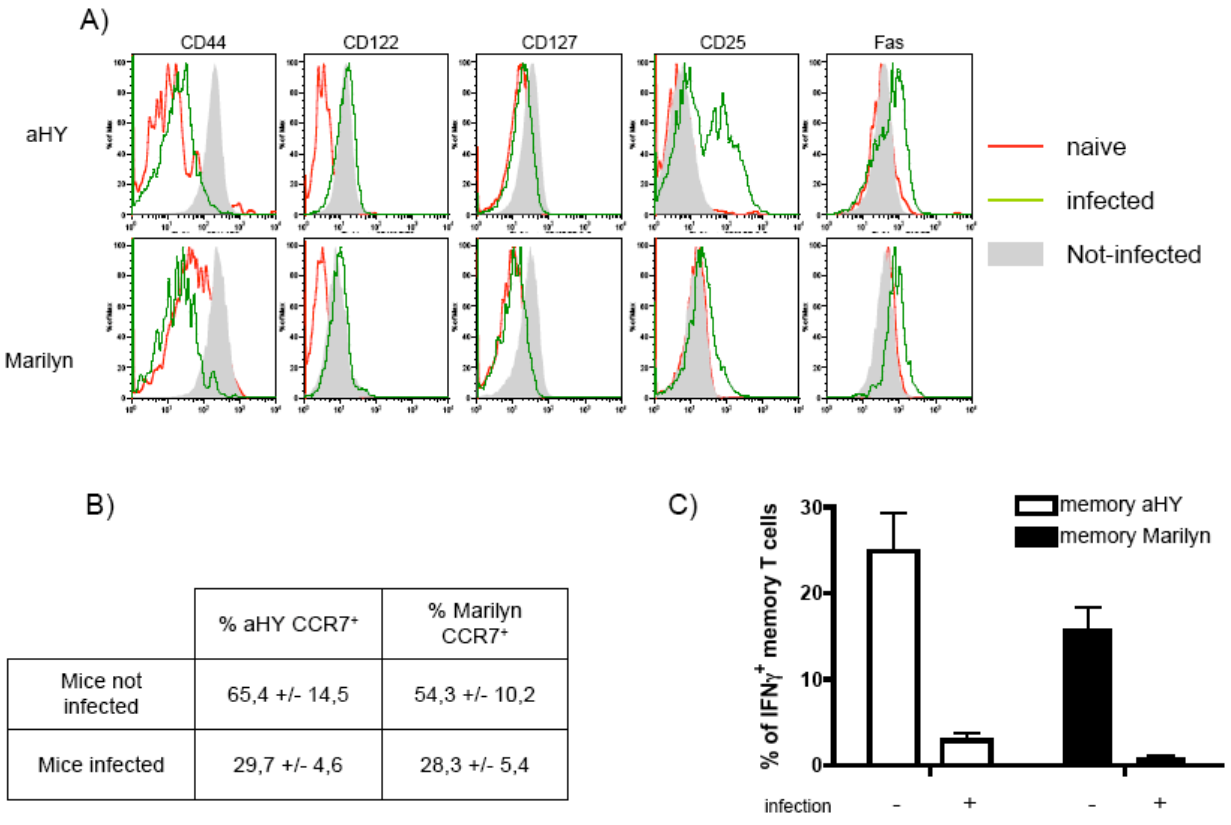
figure 7: "Behavior" of memory aHY $IFNAR^{-/-}$ and Marilyn $IFNAR^{-/-}$ upon Salmonella typhimurium infection
LNs aHY $IFNAR^{-/-}$ cells, LNs Marilyn $IFNAR^{-/-}$ cells and male BMDCs were adoptively transferred into females B6 $RAG2^{-/-}$ mice. Six weeks later, mice were i.p. injected with either PBS or Salmonella typhimurium, and 14 days upon infection, spleen was analyzed.

A) Graphic shows the number of aHY IFNAR$^{-/-}$ (white bars) and Marilyn IFNAR$^{-/-}$ (black bars) T cells when mice were infected (+) or not (-). B) Number of Salmonella Colony forming units per spleen of the different infected mice. As a control, we also determined the CFU in a non-infected mouse. C) Percentage of high expressing Bcl-2 aHY IFNAR$^{-/-}$ (white bars) and Marilyn IFNAR$^{-/-}$ (black bars) in the presence (+) or absence (-) of Salmonella infection.

Both the phenotype and function of the surviving non-specific memory T cells are impaired during Salmonella infection.

As expected, the CD8^{+} aHY and CD4^{+} Marilyn memory T cells presented higher expression levels of CD44, CD127, and CD122 than their naive counterpart (figure 8A). Expression of CD25 and Fas was low and roughly equivalent to that of naive cells (Figure 8A). However, when we compared the MFI expression of these different markers between the infected and non-infected groups, we observed that, while the CD122 expression is kept constant in both groups, in infected mice, there was an up-regulation of CD25 (especially CD8^{+} T cells) and Fas and a down-regulation of CD127 and CD44 levels. We also observed that the percentage of memory T cells belonging to the subset of CCR7^{+} T cells decreased considerably in infected mice compared to not infected (figure 8B). We also examined the function of the surviving memory T cells, analyzing the IFNγ production by 4 hours ex-vivo stimulation of these cells with PMA and ionomycin. As shown in figure 8C, we observed a dramatic decrease in the percentage of cells producing IFNγ in infected mice compared to non-infected. Although this functional defect was reproducible, its intensity was variable.

In conclusion, these results suggest that Salmonella infection affected both the phenotype and the function of the non-depleted non-specific memory T cells.



	% aHY CCR7+	% Marilyn CCR7+
Mice not infected	65,4 +/- 14,5	54,3 +/- 10,2
Mice infected	29,7 +/- 4,6	28,3 +/- 5,4

figure 8: Surface markers expression in memory aHY and Marilyn T cells

FACS MFI profile of: A) CD44, CD122, CD127 , CD25 and Fas. Each FACS profile contains three overlayed histograms to compare the MFI between the naive TCR Tg control (histogram delimited with a thick red line), the memory TCR Tg cells of non-infected mice (solid grey histogram) and the memory TCR Tg cells of infected mice (histogram delimited by a thin green line). B) Table with the percentage of $CCR7^+$ memory T cells recovered from the spleen of infected and not infected mice. C) Percentage of IFNγ producing memory T cells in infected (+) and non-infected (-) mice.

2 MATERIALS AND METHODS

Mice

B6 RAG2$^{-/-}$ mice were originally from the Centre de Distribution, Typage & Archivage Animal (CDTA Orleans, France) and were subsequently maintained as breeding colonies in our SPF animal facilities. All the TCR Tg used were also in a B6 background. AND TCR Tg RAG2$^{-/-}$ (Vα11; Vβ3 I-A^{b} restricted TCR) has T cells with specificity for pigeon cytochrome c; aHY TCR Tg RAG2$^{-/-}$ (VαT3.70; Vβ8.2 H-2D^{b} restricted TCR) and Marilyn TCR Tg RAG2$^{-/-}$ (Vα1.1; Vβ6 I-A^{b} restricted TCR) have T cells that are specific for epitopes of the H-Y antigen; and OT1 TCR Tg RAG1$^{-/-}$ (Vα2; Vβ5 H-2K^{b} restricted TCR) and OT2 RAG2$^{-/-}$ TCR Tg (Vα2; Vβ5 I-A^{b} restricted TCR) have T cells with specificity for different epitopes of the Ovalbumin protein; both OT1 and OT2 were distinguished on the basis of their Ly5.1 and Ly5.2 allelic expression.
Marilyn and aHY TCR Tg RAG2$^{-/-}$ mice were further backcrossed with B6 IFNAR$^{-/-}$, to originate TCR Tg RAG2$^{-/-}$ IFNAR$^{-/-}$ mice.
Usually we used female mice to do the experiments. The only males mice used were the B6.PL Thy1.1 and the B6 CD3ε$^{-/-}$ to produce the APCs presenting the male antigen in order to generate the memory aHY and Marilyn T cells. All mice were maintained in the SPF animal facilities under the guidelines of the Experimental Ethics Committee.

Bone marrow (BM) chimeras

BM chimeras were made by lethal irradiation (950 rads) of B6 RAG2$^{-/-}$ host female mice, and injection i.v. of 2-3x10^{6} T cell depleted BM cells from different donor mice, mixed at variable ratios. T cell depletion was performed using an AutoMACS (Milteny Biotec) after incubating BM cells with anti-CD3 biotinylated, followed by biot-MACSbeads.

Generation of memory aHY and Marilyn T cells and adoptive transfers

We used two different approaches to originate memory T cells. In one approach, along with BM coming from females OT1 and/or OT2 or AND donors, we mixed 5x10^{5} male BM CD3ε$^{-/-}$. One day after BM transfer, we adoptively transferred 5X10^{5} LN aHY and 5X10^{5} LN Marilyn T cells.

Three weeks after BM injection, male B cells are rare and undetectable thereafter (Rocha et al., 1995).

In the second approach, lymph nodes from aHY TCR Tg rag2$^{-/-}$ and Marilyn TCR Tg rag2$^{-/-}$ were adoptively transferred together with male bone marrow derived dendritic cells (BMDCs) into rag-female deficient hosts by i.v. injection. We co-transferred between 0,5x10^6 and 1x10^6 of aHY and Marilyn T cells and 1-2x10^6 male BMDCs. BMDCs were generated from 6 days culture of a male BM B6.PL Thy1.1 donor with GMCSF in a complete medium (RPMI + 10% FBS + Hepes + β-mercaptoethanol + penincilin-streptovidin) at 37°C. Male BMDCs disappear 3 weeks after transfer (Garcia et al., 1999).

In both approaches, six weeks after transfer all T cells were CD44^{hi}, were IFNγ producers upon stimulation and persisted *in vivo* in the absence of their specific antigen.

Bacteria strains

Salmonella enterica Serovar Typhimurium strains used were either χ4550-OVA or SL3261 aroA.

χ4550 contains deletions in the genes encoding adenylate cyclase and cyclic AMP receptor protein that reduces its virulence relative to wild-type strain. This bacterium is also mutant for the gene encoding aspartate β-semialdehyde dehydrogenase (ASD) and it was used as a host for the vector pYA3259 that contains the ovalbumin gene and encodes a truncated form of ASD. ASD is an enzyme in the biosynthetic pathway for diaminopimelic acid (DAP), an essential component of the peptidoglycan of the bacteria cell wall (Yrlid et al., 2001).

SL3261 aroA is a derivative of the virulent wild-type SL1344 with an extensive deletion at aroA, generated from a transposon insertion aroA554::Tn10 transductant (Hormaeche et al., 1996). Salmonella aro mutants are affected in the aromatic biosynthetic pathway, which means that they require the substrates para-aminobenzoic acid and 2,3-dihydroxybenzoate. Those substrates are not available in sufficient quantity in host tissues; therefore this strain is less virulent.

The bacteria strains were provided and prepared by the Unité de Pathogénie Microbienne Moléculaire at the Pasteur Institute.

Bacteria growth conditions

Bacteria were grown in Luria broth at 37°C with aeration. For mice infection, bacteria were ressuspended in a 0,9% NaCl solution. The concentration used for infection was calculated as follows: 1 OD at 600 nm corresponds to $2x10^9$ bacteria/ml.

Mice infection

To mimic the systemic phase of infection, bypassing the entry of the bacteria through epithelial cells in the gut, mice were i.p. infected with $1\text{-}5x10^5$ Salmonella typhimurium. Two or fourteen days upon infection, mice were sacrificed and spleen was always analyzed. Bone marrow, LNs (inguinal and mesenteric), lung and liver were also analyzed when mentioned. Control mice were i.p. injected with PBS.

Flow cytometry

Cells were stained with appropriated combinations of different antibodies purchased from either BD Pharmingen or eBioscience. The following mAbs were used: anti-CD3, anti-CD4, anti-CD8, anti-CD44, anti-CD122, anti-CD25, anti-CD45.2 (Ly5.2), anti-Fas, anti-Vα2, anti-Vβ3, anti-Vβ5 and anti-Vβ6 from BD Pharmingen; and anti-CD127, anti-VαT3.70, anti-CD4 and CD45.1(Ly5.1) from eBioscience. Cell surface staining was performed with the appropriate combinations of FITC-, PE-, PE Cy7-, Percp-, APC-, APC Alexa 750-, APC Cy7- and pacific blue- coupled antibodies.

To detect apoptotic cells, we used 7AAD (Sigma). Intracellular staining was done by culturing the cells 4h in a CO_2 incubator at 37°C in the presence of Brefeldin A (10 µg/ml), with or without PMA (50 ng/ml) plus ionomycin (500 ng/ml), followed by surface staining and fixation in 1% paraformaldehyde. Fixed cells were permeabilized with saponin (0,1%) and stained with IFNγ APC and or Bcl-2 PE. All acquisitions were done with Canto (Becton Dickinson) or CyAn (Dako) flow cytometers interfaced to the Macintosh FlowJo software (TreeStar).

Statistical analyses

Mann-Whitney test was used for data analysis. P is significant when inferior to 0,05 and ns means not significative.

DISCUSSION

DISCUSSION

As we have extensively documented in the introduction, the number of mature T cells is kept constant throughout the life by a tightly regulated process termed homeostasis. Different mechanisms contribute to the maintenance of peripheral T cell pool: thymic production, survival, proliferation and differentiation, leading to the building of two main T cell compartments according to the activation/differentiation state of the T cells; the naive and the activated/memory T cell compartments. These compartments are thought to be regulated independently. Another important event during the maintenance of the T cell pool is the occurrence of competition among T cells for limiting resources involved in the different homeostatic mechanisms. Many of these resources, mainly p-MHC and cytokines, appeared to be common to the different homeostatic processes and to the different T cell pools. During this work, we asked whether some of these resources could overlap among the different T cell pools affected by concomitant homeostatic processes, and if so, whether this overlapping may affect T cells differently according to their TCR specificity. We found that indeed, depending on the TCR specificity of the T cells, some TCR-specific resources could be shared by different T cells from different compartments and ongoing different homeostatic processes. The consequences of these findings will be discussed below. In addition, we also decided to study the influence of infection by bacteria, Salmonella typhimurium, on the T cell homeostasis, and in particular on the fate of the memory pool. Although further work will be need, preliminary data showed that this pool may be impoverished. Potential mechanisms will be discussed.

1 OVERLAP BETWEEN SURVIVAL AND LDP MECHANISMS: COMPETITION FOR P-MHC

Because survival and LDP share common resources, we decided to study if there was an overlap and interference between those two mechanisms when we forced them to occur at the same time. We, like others (Ge et al., 2004; Moses et al., 2003; Troy and Shen, 2003), have observed that, while donor TCR Tg monoclonal T cells were able to proliferate when transferred into hosts containing T cells bearing different specificity, their proliferation was totally blocked when transferred into hosts containing T cells bearing the same specificity. Therefore, this

suggested, that some of the TCR-specific resources needed by donor T cells for LDP (namely p-MHC) were used by resident T cells to survive. By comparing the recovered donor cell numbers at days 1 and 14 post- transfer of TCR Tg T cells into hosts containing T cells bearing the same specificity, we further found that, while donor T cells could not proliferate, their survival was not impaired (data not shown). Since the donor cells seemed to have sufficient amounts of resources to survive in the presence of resident T cells with the same TCR specificity, but not enough to trigger their LDP, this strongly suggests that the threshold of signaling to start T cell LDP is much higher than the one required for T cell survival.

From these experiments, it was highlighted the existence of intraclonal competition, but the question remained if this LDP inhibition could also be observed when transferring polyclonal T cells (with a wide TCR diversity) into different TCR Tg $RAG^{-/-}$ lymphopenic host mice. The aim of our study was first to investigate the existence of interclonal competition through the evidence of TCR-specific competition between resident T cell clones and polyclonal T cells, and second the influence of the size of the resident clones on the extend of this competition. We found indeed that a fraction of injected polyclonal $CD8^+$ T cells was not able to proliferate in TCR Tg hosts, although these cells could survive in the hosts since their number did not seem to decline at least over the time of the study. Interestingly, the absolute numbers of the non-dividing polyclonal donor $CD8^+$ T cells increased with the resident clone size in the periphery. At this stage, two possible hypotheses were raised to explain the existence of this non-proliferating fraction: competition with resident T cells for either TCR unspecific or specific resources. By observing that the stable fraction of cells that didn't proliferate in one specific TCR Tg host, was able to proliferate in a different TCR Tg host, even to similar extents that the ones obtained in an "empty" $RAG^{-/-}$ host, we could conclude that the limiting resources for LDP were TCR-specific.

A study made in our laboratory revealed that OT1 T cells were more promiscuous than P14 cells, that were more promiscuous than aHY. Moreover, it reported that promiscuity regulates clone size of peripheral T cells (Hao et al., 2006). These findings could explain the different numbers of polyclonal non-dividing $CD8^+$ T cells recovered in the different TCR Tg hosts. Since OT1 T cells bear more promiscuous TCRs, they are likely to interact with a wider spectrum of peptides-MHC complexes than P14 and aHY T cells, and thus, they will block a higher number of polyclonal T cells to receive enough TCR-mediated signal for activation and proliferation, than the other less promiscuous TCR bearing Tg cells. In contrast, since

promiscuity of aHY is very low, these cells only block LDP of a very few number of polyclonal T cells. Although first contradictory, our results are therefore in consensus with the report that showed that the more diverse the repertoire of resident T cells, the more the T cell proliferation is restricted (Min et al., 2004). Based on these observations, we can hypothesize that the TCR diversity of the non-divided polyclonal $CD8^+$ T cell fraction in TCR Tg OT1 hosts will be higher than in TCR Tg P14 hosts, that will be higher than in TCR Tg aHY hosts. To test this hypothesis, we will sort $CFSE^{hi}$ polyclonal $CD8^+$ T cells after transfer into the different TCR Tg hosts and we will compare their diversity by sequencing their TCRs.

During the polyclonal $CD8^+$ T cell adoptive transfers, the main percentage of T cells had a naive phenotype (90%) before the adoptive transfer. Therefore, we hypothesized that maybe the fraction of undivided T cells corresponded to only naive $CD8^+$ T cells that needed the same p-MHC interactions for LDP as the resident naive T cells were consuming to survive. We found that memory T cells LDP was also inhibited by the resident naive T cells and were, thus, as susceptible as naive T cells to the inaccessibility to appropriate p-MHC ligands to proliferate. Nevertheless, for a same specificity, it is probable that the threshold of activation for naive T cell LDP is higher than that required for memory T cell LDP. Although we showed that the overlap of survival of resident naive T cells and LDP of both naive and memory T cells was mainly due to competition for p-MHC, we can not rule out the importance of cytokines in our experiments.

Our results indicated that some transferred polyclonal T cells could not proliferate because resident monoclonal T cells were competing for the same specific resources to survive and did not allow them to get enough p-MHC signals to trigger division. We then asked what would happen if resident and transferred populations were co-transferred in a same ratio into a lymphopenic host. This experiment indeed demonstrated that the polyclonal $CD8^+$ T cells that were not able to proliferate in a TCR Tg host, out-competed the resident T cells from that host for LDP. This result, therefore, allows us to more strongly support the idea that there is an overlap between those two mechanisms. When both cell types are "submitted" at the same time to LDP, the cells that have a better fitness will out-compete the others, in that case the polyclonal T cells, probably because of their overall greater TCR promiscuity (Hao et al., 2006).

Therefore, the sharing of common resources, especially p-MHC between T cells subjected to different homeostatic processes and belonging to different compartments, modulates the previously proposed independency of the two T cell pools (Tanchot and Rocha, 1997).

In the previous experiments, the resident T cells always blocked to some extend the establishment of the transferred polyclonal population, following the rule "first come, first served". We wanted then to study whether the same rule was still applied when the resident population was not naive but memory and when the introduced subset was mature (adoptive transfer) or derived from BM-progenitors (BM-engraftment), and if so, whether it was related to TCR specific resources.

We analyzed different situations, summarized in the table below:

	RESIDENT SUBSET	**NEWLY INTRODUCED SUBSET**
1	LDP-derived	Naive adoptively transferred
2	LDP-derived	BM-derived
3	True-memory	BM-derived

In the first two situations (1 and 2), we first injected $RAG^{-/-}$ host with a mature monoclonal $CD8^+$ T cell population (either it was OT1 or P14 T cells), followed 4 weeks after (when the LDP-derived T cells had reached a plateau) with a second subset. When this second transfer consisted on mature monoclonal $CD8^+$ T cells bearing the same or a different specific TCR (1), the accumulation of the second injected population was reduced in a non-specific manner, although we observed a delayed CFSE proliferation pattern dependent on TCR specificity, consistent with what was previously reported (Ge et al., 2004). These results suggest that the final T cell number recovered may be reached through two phases: first, a division process, which likely require TCR/p-MHC interactions, and is submitted to competition with identical resident clones, although less dramatically than when the later is naive likely due to a lower requirement of memory T cells on p-MHC; and second an accumulation process of the dividing cells, which may involve competition for non-TCR-specific resources, such as IL-7 or IL-15, and dictate the final number of the second population of LDP-derived T cells.

When the second introduced T cell subset derived from BM-precursors (2), the number of BM-derived T cells was significantly decreased in the presence of LDP-derived T cells bearing the same TCR specificity, independently of whether they belonged to the naive or activated/memory

pool. Importantly, the thymic development was not affected by the presence of resident T cells, further emphasizing the homeostatic independency between thymic development and peripheral pools (Berzins et al., 1998; Berzins et al., 1999; Almeida et al., 2001;). Therefore, TCR-specific resources required for the accumulation of BM-derived T cells (involving either survival and/or LDP after colonization of the periphery in the lymphopenic environment of the hosts) is impaired by the presence of the same LDP-derived clones, suggesting again that the later interact continuously with p-MHC to maintain. Here again, the final equilibrium followed the rule "first come, first served".

The fact that LDP-derived resident T cells have a different impact on mature transferred T cells versus recent developed T cells coming from BM precursors suggests that these two types of populations have different competitive fitness. Alternatively, we cannot exclude that it is the result of a unfair competitive situation involving continuous T cell production in the BM transfer compared to a limited T cell numbers in the adoptive transfer.

Importantly, in both approaches 1 and 2 the number of LDP-derived resident T cells was not affected by the incoming of new cells, whenever they were transferred or BM-derived.

Finally, we analyzed a third situation in which we used "true" memory T cells as resident T cells (3). We made a chimeric mouse, that consisted of $RAG^{-/-}$ hosts containing both memory anti-male TCR Tg $CD8^+$ and $CD4^+$ T cells, class I (aHY) and class II (Marylin) restricted, engrafted with BM precursors from both anti-OVA TCR Tg $CD4^+$ (OT2 strain) and $CD8^+$ (OT1 strain). We found a 2 to 6 fold decrease of the number of the resident aHY memory $CD8^+$ T cells and much less reproducible, a 2-4 fold decrease of the resident Marylin memory $CD4^+$ T cells. By analogy with the attrition process described during microbial infections and developed in the introduction part (chapter 3.3, p49), we named this process "natural" attrition of the memory T cells. Using BM-transfer of OT1 or OT2 BM-precursors alone, we found that only OT1 BM-derived T cells were able to induce the depletion of some resident T cells. Because the number of peripheral OT2 T cells were far less important than that of OT1 T cells (very likely due to their TCR promiscuity difference), we decided to test whether BM-derived $CD4^+$ T cells from the promiscuous class II-restricted anti-PCC TCR Tg strain AND, could displace the resident $CD8^+$ T cell memory. We found that indeed they could, indicating that TCR promiscuity and subsequent T cell clone size of BM-derived T cells was much more relevant in that process of natural attrition of resident memory than their lineage. To further test this hypothesis, we will

invert the system, by engrafting aHY BM-precursors into $RAG^{-/-}$ hosts containing OT1 resident memory T cells. If aHY newly produced T cells are not able to displace memory OT1 T cells, this will argue that memory displacement by newly produced T cells depends on the TCR degeneracy of the later.

2 INTERDEPENDENCE BETWEEN NAIVE AND MEMORY T CELL COMPARTMENTS

The fact that memory-like T cells interfere with the accumulation of BM-derived T cells (either with naive or memory phenotype) in a TCR-dependent manner, and that in other cases, BM-derived T cells with a naive phenotype are able to displace resident memory T cells question the precedent statement of independent homeostatic regulation of naive and memory T cells. This statement was first based on the observation that BM-derived polyclonal T cells affected resident naive aHY T cells, while memory aHY T cells remained unaffected (Tanchot and Rocha, 1997). Nevertheless, when one looks carefully these data, while the effect on naive T cells is far more dramatic than on memory T cells, the later are still displaced although in a smaller proportion than naive T cells (about 5 times less). In another study, it was showed that TCR Tg $CD4^+$ T cells with a memory phenotype (either due to LDP or to activation with the nominal antigen) were displaced by polyclonal BM-derived T cells (Bourgeois et al., 2005). Nevertheless, this displacement was explained as being due to the action of the $CD44^{hi}$ BM derived T cells, and thus also arguing for the independent regulation of the naive and memory T cell pool (although without any experimental evidence).

If the original statement remains globally true, our data showed that it had to be modulated. They may lead to two main considerations:

-The first one is that the system may avoid a redundancy of specificity by inhibiting the establishment of new T cells bearing the same specificity than resident T cells, whenever they belong to the naive or memory T cell pool. Intriguingly, this was not observed in the case of A1 TCR Tg $CD4^+$ T cells, since BM-derived A1 T cells are able to establish in the presence of memory A1 T cells (Bourgeois et al., 2005). Whether it reflects a difference between $CD4^+$ and $CD8^+$ T cell behavior remains to be determined.

-The second one is that memory T cell displacement by new generated naive T cells may depend on the clonal size of the naive T cells in the periphery and thus on their TCR degeneracy.

Nevertheless, these two points have not the same impact since the first one leads to the total block of the accumulation of new produced T cells, while the second one leads to a much more modest effect, in particular when compared with the effect on naive resident T cells observed by others (Tanchot and Rocha, 1997). While the first mechanism implicates competition for p-MHC ligands, the mechanism beneath the "natural" attrition remains to be characterized. What can be concluded from our data is that it depends on the clone size of the BM-derived T cells, as discussed above, and that it does not depend on the lineage of the BM-derived T cells. Because the only common resource to both $CD4^+$ and $CD8^+$ subsets and to both naive and memory T cells is the IL-7 (Vella et al., 1997; Boursalian and Bottomly, 1999; Schluns et al., 2000; Tan et al., 2001; Vivien et al., 2001; Seddon et al., 2003; Kondrack et al., 2003; Li et al., 2003;), the natural candidate to explain this observed natural attrition is this cytokine. The differential documented role of IL-7 in the survival of $CD8^+$ and $CD4^+$ memory T cells may explain our finding that memory $CD8^+$ aHY T cells are more susceptible to natural attrition than memory $CD4^+$ Marilyn T cells. Although the monoclonal OT1 and AND T cells express the same or slightly lower level of CD127 (the α chain of IL-7 receptor) (data not shown) compared to the memory $CD8^+$ aHY T cells, their higher TCR degeneracy may confer them better competitive capacities for IL-7 and/or a better quality of IL-7R signaling. Alternatively, other possible reason for the non-reproducibility of $CD4^+$ Marilyn memory T cell deletion could be related with the fact that the numbers of the $CD4^+$ memory subset was (even in the control group) usually much lower than the $CD8^+$ memory subset. And therefore, when the frequency of memory T cells is more rare, those cells are in terms of probability more "protected" from deletion.

In summary, our data led to the conclusion that naive and memory T cell compartments are not totally independent. The sharing of some resources, p-MHC/TCR interactions and cytokines, namely IL-7, common factors for survival of the two pools (naive and activated/memory pools), would be responsible for, at least, a partial interdependent homeostatic regulation. Nevertheless, naive and activated/memory T cells occupy different niches that partially overlap, and as a consequence, in general, memory T cells affect much more other memory T cells than naive T cells and naive T cells have a higher impact in other naive T cells than in memory T cells.

3 IMPORTANCE OF TCR DEGENERACY IN PERIPHERAL HOMEOSTASIS AND CONSEQUENCE FOR THE T CELL POOL

Altogether, these data show the occurrence of competition among T cells that are subjected to different homeostatic processes, and (which is more contradictory with previous statements) among T cells belonging to different T cell compartments. T cells compete for p-MHC ligands and also probably for non-specific ligands, cytokines, although our study did not directly demonstrate it. This competition is more often detrimental for new establishing cells, at least in the systems we decided to study, confirming the rule "first come, first served". It is the case for mature T cells which cannot divide (in spite of the relative lymphopenia of the hosts) when naive T cells from the same specificity or recognizing same p-MHC ligands are already established. The "first come, first served" rule was also observed for resident activated/memory T cell clones, which inhibit the settlement of new BM-derived T cell bearing the same TCR. This competition for p-MHC ligands, may avoid redundancies in the T cell repertoire in the case of the same clones, favoring a larger diversity of Ag recognition. It might be more valuable for the organism to keep a diverse naive repertoire, on the one hand, and to keep faster responders activated/memory T cells instead of slower responder naive T cells.

The fact that it affects T cells from different T cell compartment may imply that, to a certain extend, the T cell repertoire is build as a "whole", including T cells from different origin (recent thymic emigrant, resident naïve, resident activated/memory, resting or dividing). The situation is quite similar when the new introduced T cells population transferred mature T cells, although in that case it is also true when both populations are from different specificity, involving competition for non-TCR specific resources. This competitive mechanism may in part restrain the size of the LDP-activated pool, maybe avoiding the occurrence of hazardous auto-immunity.
However, this "first come, first served" rule does not apply in all situations when T cells have to compete for resources. Indeed, although we did not explore this situation, it has been clearly demonstrated that new BM-derived T cells could displace resident naive T cells but in a non-TCR-specific manner (Tanchot and Rocha, 1997). We also showed that in some cases when the TCR degeneracy of the new establishing T cell population is much higher than that of the resident T cells, naive BM-derived T cells could displace activated/memory T cells, what we named "natural attrition" by opposition with the well described "attrition" reported during microbial infections.

Therefore, a major component of competition during T cell homeostasis seems to be the properties of a defined TCR to be degenerated and to recognize a large panel of p-MHC ligands. This capacity confer to the cells bearing such TCRs much higher competitive capacities as it has been shown throughout this work and by other studies (Kassiotis et al., 2003; Bourgeois et al., 2005; Hao et al., 2006). Indeed, as it dictates the size of a clone, we may have to consider not the diversity of the repertoire as the diversity of different clones per se, but as the repertoire of p-MHC ligands and further Ag that can be recognized by the T cell pools.

4 ANOTHER CASE: MEMORY ATTRITION INDUCED BY INFECTION

While the above findings tried to reproduce what may occur in steady state situations, in absence of intentional immunization, we then asked what might happen to the memory pool during a bacterial infection.

The term of memory T cell "attrition" was first applied to explain the changes in frequency and dominance of memory T cells upon sequential mice infection with several heterologous viruses (Selin et al., 1999). Since then, several studies have been performed in an attempt to find if attrition can also be observed in infections with other pathogens, if there is a selective process of attrition, when does it occur and what is the mechanism involved in this process. Although much work has been done, there are still much more to do in order to completely understand attrition mediated by infection. We thus decided to study this process in an infectious model never used before for this purpose, namely the infection by Salmonella thyphimurium. We used an attenuated strain of bacteria, because the virulent one induces B6 mice death much before the generation of adaptative response, which would interfere with our aim to study T cell fate.

Attrition: selective versus non-selective process

It is still not known whether attrition previously described is a selective or non-selective process. Among the parameters of potential selectivity, there are:

a) $CD8^+$ vs $CD4^+$ T cells; most of the studies have shown $CD8^+$ T cell deletion (Selin et al., 1999; McNally et al., 2001; Varga et al., 2001; Kim et al., 2002; Smith et al., 2002; Chapdelaine et al., 2003; Peacock et al., 2003; Kim and Welsh, 2004;). Only one report

showed $CD4^+$ T cell deletion (Smith et al., 2002), but this observation was made during chronic infection with *Mycobacterium bovis.*

b) memory vs naive T cells; most of the studies that have shown $CD8^+$ T cell deletion have focused on memory T cell attrition; only some reports have shown that attrition can also be observed in naive T cells (Jiang et al., 2003b; McNally et al., 2001; Smith et al., 2002).
c) specific vs non-specific T cells; one report documented attrition of only pathogen non-specific T cells (Jiang et al., 2003b), whereas other report showed a general attrition of both pathogen specific and non-specific T cells (Bahl et al., 2006).
d) T cell function (Th1, Th2, Th17…); will be investigated if we observe that the attrition observed is specific to T cells.

Although preliminary, our experimental model, which consisted of chimeric mouse reconstituted by both $CD8^+$ aHY and $CD4^+$ Marilyn T cells that recognize the male antigen and do not recognize Salmonella Ag (data not shown), allowed us to answer the first point. The choice of Salmonella typhimurium as the pathogenic agent in our study was related to the fact that attrition had only been demonstrated in viral (Selin et al., 1999; Selin et al., 1996) and intracellular bacteria (Jiang et al., 2003b; Bahl et al., 2006) infections, but never with a facultative intracellular bacteria that, in opposition to the other types of infection, induces a very strong $CD4^+$ T cell response (Srinivasan et al., 2004). Thus, our aim was to determine if a pathogen with this kind of features could also induce $CD4^+$ T cell depletion that was not observed in the other infections (Varga et al., 2001).

Salmonella's intra-peritoneal infection induced attrition of both non-specific $CD8^+$ and $CD4^+$ memory T cells. Therefore, in our system, it seemed that attrition did not affect selectively $CD8^+$ T cells, but also $CD4^+$ T cells. Nevertheless, the level of attrition was higher for memory $CD8^+$ T cells than $CD4^+$ T cells. To note, the experiments were initially done by using the attenuated χ4550-OVA strain, but the later use of another Salmonella non-transgenic attenuated strain (SL3261), which gave the same results, showed that the χ4550-OVA strain did not bias our experiments.

Because we observed, with our system, attrition of non-specific memory T cells in a reproducible way, we next want to test whether naive T cells are also susceptible to attrition. For this, we will create BM chimera by engraftment of a mixture of precursors from both aHY and

Marylin donors. We want to determine if we can also observe attrition in non-specific naive T cells, and if we observe, we want to compare the extension of their attrition with the "memory" chimeric mice.

In order to have a clue about specific vs non-specific T cells attrition, we will infect chimeric mice containing both the non-specific anti-male memory T cells and the SM1 TCR Tg mice, that bear an anti-Salmonella flagelin I-A^b restricted-TCR (McSorley et al., 2002), and compare the fate of each subset. Alternatively, we will re-activate *in vivo* aHY and Marilyn memory T cells by transfer of male BMDCs and infect the mice immediately after. The potential resistance of re-activated memory T cells versus non-re-activated memory T cells to attrition will be then evaluated.

We still cannot answer if this death is not a general bacteria toxic effect, although nobody reported any cell death excepted the infected macrophages and DCs (Fink and Cookson, 2007).

5 ATTRITION: CELL MIGRATION INTO OTHER ORGANS VERSUS APOPTOSIS

Attrition is a common feature of many severe viral and bacterial infections, that occurs prior to the peak of T cell response (Jiang et al., 2003b; Peacock et al., 2003; Reed et al., 2004). Some reports have shown that this attrition was a consequence of cell death (McNally et al., 2001; Jiang et al., 2003b; Bahl et al., 2006), whereas others demonstrated, that it was a consequence of lymphocytes redistribution (Gresser et al., 1981; Schattner et al., 1983).

The comparative analyses of memory T cell numbers from different lymphoid and non lymphoid organs of Salmonella-infected versus non-infected mice allowed us to conclude that in our system, attrition is a consequence of death of the memory T cells rather than a re-localization of these cells out of the spleen. The study of the apoptotic status of both $CD4^+$ and $CD8^+$ anti-male memory T cells after infection supported this conclusion.

By using $TRAIL^{-/-}$ mice and mice treated with soluble death receptor 5, attrition by apoptotic death during infection with *Listeria monocytogenes* has been suggested to be induced via TNF-related-apoptosis-inducing ligand (TRAIL) (Jiang et al., 2003b). These observations tend to support an extrinsic apoptotic pathway. Although we did not look at the expression of this receptor during Salmonella infection, we observed the increased expression of another death receptor, Fas. The use of $Fas^{-/-}$ T cells should help to understand the involvement of this molecule

in Salmonella-induced death of memory T cells. In addition, we have evidenced that memory T cells from infected mice showed a decrease in Bcl-2 levels comparing to non-infected mice, suggesting a protective role of Bcl-2 in the apoptosis mechanisms involved during Salmonella infection. We are currently confirming these data by using memory T cells from aHY and Marilyn donors that we have crossed with mice transgenic for the human Bcl-2 molecule (Strasser et al., 1991; Akashi et al., 1997). Since caspase-8 is a major initiator caspase, which is activated upon death receptor signaling and can initiate the activation of the caspase cascade via both the death receptor and mitochondrial death pathways (Li et al., 1998; Luo et al., 1998; Yin et al., 1999), it would be also of interest to study its activation in memory T cells in our experimental model.

6 POSSIBLE MECHANISMS OF ATTRITION

Due to the construction of our chimeric mice, we can tell that T cell memory attrition can occur in the absence of competition with other T cells, suggesting other mechanisms than competition for surviving resources such as IL-7 or IL-15 during Salmonella infection.

Some reports have observed that attrition occurs in the early phase of infection, like day 2 after infection or poly(I:C) injection (McNally et al., 2001; Jiang et al., 2003a; Jiang et al., 2003b; Kim and Welsh, 2004; Jiang et al., 2005b; Bahl et al., 2006). These observations led to hypothesize that this attrition was related with the release of inflammatory cytokines, mediated by activated cells of the innate immune system (Selin et al., 2004), for example, type I interferon. Indeed, some reports, by using mice deficient for IFNα/β receptor ($IFNAR^{-/-}$), demonstrated that in those mice, T cells were more resistant to apoptosis than in wild type mice (McNally et al., 2001; Carrero et al., 2004; Selin et al., 2004). Therefore, we decided to look as early as day 2 and at day 14 after Salmonella infection to study if we observed a similar early T cell depletion. In our case we did not get an early T cell deletion, since we only obtained it at day 14 post-infection. However, at day 2 the number of CFU/spleen was inexistent or very small compared to day 14. Therefore the very weak bacterial load may not have induced a release of inflammatory molecules high enough to induce attrition. Another explanation that is not mutually exclusive is the use of two strains of attenuated Salmonella bacteria, which have likely altered kinetics of bacterial expansion early after infection. Altogether, these results suggest a role of bacterial load, direct or indirect, in the T cell memory attrition induced during Salmonella infection.

Since Salmonella typhimurium stimulates type I interferon synthesis after the invasion of the cell (Bogdan et al., 2004), we decided to determine if IFNα/β played a role in attrition, like it had been suggested for other infections (McNally et al., 2001; Carrero et al., 2004; Selin et al., 2004). By generating chimeric mice constituted with aHY IFNAR$^{-/-}$ and Marilyn IFNAR$^{-/-}$ memory T cells, we first decided to investigate the potential direct role of IFNα/β on memory T cells during Salmonella-induced attrition. Importantly, we did not find in our system any functional defect of aHY IFNAR$^{-/-}$ and Marilyn IFNAR$^{-/-}$ memory T cells in terms of proliferation, and function (same numbers of anti-male memory T cells obtained whatever their expression of IFNAR, and same production of IFNγ), in contrast to what was observed during LCMV infection (Kolumam et al., 2005). Because aHY IFNAR$^{-/-}$ and Marilyn IFNAR$^{-/-}$ memory T cells presented the same level of attrition than wild type memory T cells, we concluded, that IFNα/β did not have any direct role on T cell depletion. To note, the absence of a role of IFNα/β may be a feature of Salmonella's infection. Indeed, while type I interferons are required for activation of the inflammasome and for the cell death of macrophages during Francisella infection, Salmonella typhimurium-mediated macrophage cell death appears to be type I IFN-independent (Henry et al., 2007). Nevertheless, we cannot exclude an indirect role of type I interferon through its action in cells from the innate immune system that expressed the IFNα/β receptor in our system. Thus, our next experiment will be to use RAG$^{-/-}$ IFNAR$^{-/-}$ hosts containing wild type memory T cells to confirm or not the role of IFNα/β in Salmonella-induced attrition of memory T cells.

CD8^{+} aHY and CD4^{+} Marilyn memory T cells showed altered phenotype pattern and function cell impairment (in terms of IFNγ production) after infection with Salmonella as compared with non-infected mice. Among the phenotypic characteristic of the non-specific memory T cells that we observed in infected mice, we observed a very strong decrease of the expression levels of the CD44 activation marker similar to what is commonly obtained for naive T cells.

CD44 is a type I transmembrane glycoprotein. This cell-adhesion molecule is ubiquitously expressed on the surface of leukocytes, parenchymal cells and keratinocytes (Pure and Cuff, 2001). The principal known ligand for CD44 is the glycosaminoglycan hyaluronic acid (HA), a major constituent of extracellular matrices (Hughes et al., 1981; Trowbridge et al., 1982; Culty et al., 1990; Miyake et al., 1990;). Several reports suggest an inflammatory role for CD44 (Brocke

et al., 1999; Camp et al., 1993; Rafi-Janajreh et al., 1999; Pure and Cuff, 2001; Wang et al., 2002), although this effect is controversial (Teder et al., 2008; Jiang et al., 2005a; Kawana et al., 2002). Moreover, in models of inflammatory colitis in mice with targeted deletions of exon 7, or exons 6 and 7 of CD44, a reduction in disease activity was associated with an increase in apoptosis of infiltrating cells (Wittig et al., 2000). Thus, CD44 might regulate cell survival of leukocytes during evolution of an inflammatory response. To determine whether CD44 reduction expression in our system is related to cell death, it would be of interest to compare the survival capacity of "CD44-deficient" and wild-type T cells upon Salmonella infection. In addition, a very recent paper has demonstrated that CD44 is responsible for suppression of TLR-mediated inflammation (Kawana et al., 2008), because of its regulatory effect on TLR signaling through the inhibition of NF-κB activation in BM macrophages after TLR-ligand stimulation.

7 FUTURE PERSPECTIVES

Many questions are still waiting to be answered concerning T cell homeostasis and involved T cell competition; we have risen in detail all along this discussion. Concerning what we called "natural attrition", we still have to play with different TCR specificity to further analyze the impact of TCR degeneracy on the better fitness of BM-derived T cells, and the mechanisms associated with this attrition. Concerning the attrition induced by Salmonella infection, we have to better define whether this observation is applied to all cells of the infected-organism, or characteristic of non-specific memory T cells, and not a particular feature of these anti-male T cells. For this, we will make use of different approaches, involving transgenic or knocked out mice for different populations. Here again, if we find a specific effect on non-anti-Salmonella memory T cells, we will first try to determine the mechanism of this attrition, again using KO mice, or transfection technology by vector encoding for siRNA specific for potential targets like TLRs in order to modify our memory T cells. The eventual focus on TLRs as an eventual mechanism of T cell attrition will only be done if we find TLR expression on aHY and Marilyn T cells, and this will be tested by single cell qPCR. Besides the complementary experiments proposed throughout the discussion, it would be interesting to define whether all

memory T cells are equally susceptible to this attrition in terms of 1) TCR specificity, and in particular TCR promiscuity; 2) differentiation status after activation (T_{CM} vs T_{EM}); 3) LDP vs nominal Ag activation; 4) effector functions (Th1, Th2, Th17…).

Altogether, these data contribute to show the elements, which participate in the shaping of the final T cell repertoire. This shaping may be reformatted at steady state but also after infections. This work may help us to understand the final establishment of the T cell pool and of the final TCR repertoire.

In a more applied perspective, unraveling the mechanisms underlying attrition upon infection is of major interest not only in the fundamental perspective but also in a practical point of view. By discovering those mechanisms, doors can be opened in the future to develop therapies to try to contradict the trend of losing memory T cells, specially in chronic infected patients (like HIV infected individuals).

BIBLIOGRAPHY

BIBLIOGRAPHY

A

Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R., and Weissman, I.L. (1997). Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. Cell *89*, 1033-1041.

Almeida, A.R., Borghans, J.A., and Freitas, A.A. (2001). T cell homeostasis: thymus regeneration and peripheral T cell restoration in mice with a reduced fraction of competent precursors. The Journal of experimental medicine *194*, 591-599.

Appay, V., Dunbar, P.R., Callan, M., Klenerman, P., Gillespie, G.M., Papagno, L., Ogg, G.S., King, A., Lechner, F., Spina, C.A.*, et al.* (2002). Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nature medicine *8*, 379-385.

Ashton-Rickardt, P.G., and Tonegawa, S. (1994). A differential-avidity model for T-cell selection. Immunology today *15*, 362-366.

B

Bachmann, M.F., Barner, M., Viola, A., and Kopf, M. (1999a). Distinct kinetics of cytokine production and cytolysis in effector and memory T cells after viral infection. European journal of immunology *29*, 291-299.

Bachmann, M.F., Gallimore, A., Linkert, S., Cerundolo, V., Lanzavecchia, A., Kopf, M., and Viola, A. (1999b). Developmental regulation of Lck targeting to the CD8 coreceptor controls signaling in naive and memory T cells. The Journal of experimental medicine *189*, 1521-1530.

Badovinac, V.P., Haring, J.S., and Harty, J.T. (2007). Initial T cell receptor transgenic cell precursor frequency dictates critical aspects of the CD8(+) T cell response to infection. Immunity *26*, 827-841.

Badovinac, V.P., Porter, B.B., and Harty, J.T. (2002). Programmed contraction of CD8(+) T cells after infection. Nature immunology *3*, 619-626.

Bahl, K., Kim, S.K., Calcagno, C., Ghersi, D., Puzone, R., Celada, F., Selin, L.K., and Welsh, R.M. (2006). IFN-induced attrition of CD8 T cells in the presence or absence of cognate antigen during the early stages of viral infections. J Immunol *176*, 4284-4295.

Baron, V., Bouneaud, C., Cumano, A., Lim, A., Arstila, T.P., Kourilsky, P., Ferradini, L., and Pannetier, C. (2003). The repertoires of circulating human CD8(+) central and effector memory T cell subsets are largely distinct. Immunity *18*, 193-204.

Bassiri, H., and Carding, S.R. (2001). A requirement for IL-2/IL-2 receptor signaling in intrathymic negative selection. J Immunol *166*, 5945-5954.

Becker, T.C., Wherry, E.J., Boone, D., Murali-Krishna, K., Antia, R., Ma, A., and Ahmed, R. (2002). Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. The Journal of experimental medicine *195*, 1541-1548.

Bender, J., Mitchell, T., Kappler, J., and Marrack, P. (1999). CD4+ T cell division in irradiated mice requires peptides distinct from those responsible for thymic selection. The Journal of experimental medicine *190*, 367-374.

Berard, M., and Tough, D.F. (2002). Qualitative differences between naive and memory T cells. Immunology *106*, 127-138.

Berg, L.J., Pullen, A.M., Fazekas de St Groth, B., Mathis, D., Benoist, C., and Davis, M.M. (1989). Antigen/MHC-specific T cells are preferentially exported from the thymus in the presence of their MHC ligand. Cell *58*, 1035-1046.

Berzins, S.P., Boyd, R.L., and Miller, J.F. (1998). The role of the thymus and recent thymic migrants in the maintenance of the adult peripheral lymphocyte pool. The Journal of experimental medicine *187*, 1839-1848.

Berzins, S.P., Godfrey, D.I., Miller, J.F., and Boyd, R.L. (1999). A central role for thymic emigrants in peripheral T cell homeostasis. Proceedings of the National Academy of Sciences of the United States of America *96*, 9787-9791.

Bogdan, C., Mattner, J., and Schleicher, U. (2004). The role of type I interferons in non-viral infections. Immunological reviews *202*, 33-48.

Borowski, A.B., Boesteanu, A.C., Mueller, Y.M., Carafides, C., Topham, D.J., Altman, J.D., Jennings, S.R., and Katsikis, P.D. (2007). Memory CD8+ T cells require CD28 costimulation. J Immunol *179*, 6494-6503.

Bosco, N., Agenes, F., and Ceredig, R. (2005). Effects of increasing IL-7 availability on lymphocytes during and after lymphopenia-induced proliferation. J Immunol *175*, 162-170.

Bouneaud, C., Garcia, Z., Kourilsky, P., and Pannetier, C. (2005). Lineage relationships, homeostasis, and recall capacities of central- and effector-memory CD8 T cells in vivo. The Journal of experimental medicine *201*, 579-590.

Bourgeois, C., Kassiotis, G., and Stockinger, B. (2005). A major role for memory CD4 T cells in the control of lymphopenia-induced proliferation of naive CD4 T cells. J Immunol *174*, 5316-5323.

Bourgeois, C., Rocha, B., and Tanchot, C. (2002). A role for CD40 expression on CD8+ T cells in the generation of CD8+ T cell memory. Science (New York, N.Y *297*, 2060-2063.

Boursalian, T.E., and Bottomly, K. (1999). Survival of naive CD4 T cells: roles of restricting versus selecting MHC class II and cytokine milieu. J Immunol *162*, 3795-3801.
Boyman, O., Ramsey, C., Kim, D.M., Sprent, J., and Surh, C.D. (2008). IL-7/Anti-IL-7 mAb Complexes Restore T Cell Development and Induce Homeostatic T Cell Expansion without Lymphopenia. J Immunol *180*, 7265-7275.

Braddock, M., Quinn, A., and Canvin, J. (2004). Therapeutic potential of targeting IL-1 and IL-18 in inflammation. Expert opinion on biological therapy *4*, 847-860.

Brady, H.J., Gil-Gomez, G., Kirberg, J., and Berns, A.J. (1996). Bax alpha perturbs T cell development and affects cell cycle entry of T cells. The EMBO journal *15*, 6991-7001.

Brocke, S., Piercy, C., Steinman, L., Weissman, I.L., and Veromaa, T. (1999). Antibodies to CD44 and integrin alpha4, but not L-selectin, prevent central nervous system inflammation and experimental encephalomyelitis by blocking secondary leukocyte recruitment. Proceedings of the National Academy of Sciences of the United States of America *96*, 6896-6901.

Brocker, T. (1997). Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expressing dendritic cells. The Journal of experimental medicine *186*, 1223-1232.

Brunner, T., Mogil, R.J., LaFace, D., Yoo, N.J., Mahboubi, A., Echeverri, F., Martin, S.J., Force, W.R., Lynch, D.H., Ware, C.F., and et al. (1995). Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. Nature *373*, 441-444.

C

Camp, R.L., Scheynius, A., Johansson, C., and Pure, E. (1993). CD44 is necessary for optimal contact allergic responses but is not required for normal leukocyte extravasation. The Journal of experimental medicine *178*, 497-507.

Capone, M., Hockett, R.D., Jr., and Zlotnik, A. (1998). Kinetics of T cell receptor beta, gamma, and delta rearrangements during adult thymic development: T cell receptor rearrangements are present in CD44(+)CD25(+) Pro-T thymocytes. Proceedings of the National Academy of Sciences of the United States of America *95*, 12522-12527.
Carrero, J.A., Calderon, B., and Unanue, E.R. (2004). Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to Listeria infection. The Journal of experimental medicine *200*, 535-540.

Catalina, M.D., Sullivan, J.L., Brody, R.M., and Luzuriaga, K. (2002). Phenotypic and functional heterogeneity of EBV epitope-specific CD8+ T cells. J Immunol *168*, 4184-4191.

Catron, D.M., Rusch, L.K., Hataye, J., Itano, A.A., and Jenkins, M.K. (2006). CD4+ T cells that enter the draining lymph nodes after antigen injection participate in the primary response and become central-memory cells. The Journal of experimental medicine *203*, 1045-1054.

Chalker, R.B., and Blaser, M.J. (1988). A review of human salmonellosis: III. Magnitude of Salmonella infection in the United States. Reviews of infectious diseases *10*, 111-124.

Champagne, P., Ogg, G.S., King, A.S., Knabenhans, C., Ellefsen, K., Nobile, M., Appay, V., Rizzardi, G.P., Fleury, S., Lipp, M.*, et al.* (2001). Skewed maturation of memory HIV-specific CD8 T lymphocytes. Nature *410*, 106-111.

Chandele, A., and Kaech, S.M. (2005). Cutting edge: memory CD8 T cell maturation occurs independently of CD8alphaalpha. J Immunol *175*, 5619-5623.

Chao, D.T., and Korsmeyer, S.J. (1998). BCL-2 family: regulators of cell death. Annual review of immunology *16*, 395-419.

Chapdelaine, Y., Smith, D.K., Pedras-Vasconcelos, J.A., Krishnan, L., and Sad, S. (2003). Increased CD8+ T cell memory to concurrent infection at the expense of increased erosion of pre-existing memory: the paradoxical role of IL-15. J Immunol *171*, 5454-5460.

Cheng, L.E., Ohlen, C., Nelson, B.H., and Greenberg, P.D. (2002). Enhanced signaling through the IL-2 receptor in CD8+ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. Proceedings of the National Academy of Sciences of the United States of America *99*, 3001-3006.

Cho, J.H., Boyman, O., Kim, H.O., Hahm, B., Rubinstein, M.P., Ramsey, C., Kim, D.M., Surh, C.D., and Sprent, J. (2007). An intense form of homeostatic proliferation of naive CD8+ cells driven by IL-2. The Journal of experimental medicine *204*, 1787-1801.

Ciurea, A., Klenerman, P., Hunziker, L., Horvath, E., Odermatt, B., Ochsenbein, A.F., Hengartner, H., and Zinkernagel, R.M. (1999). Persistence of lymphocytic choriomeningitis virus at very low levels in immune mice. Proceedings of the National Academy of Sciences of the United States of America *96*, 11964-11969.

Clarke, S.R., and Rudensky, A.Y. (2000). Survival and homeostatic proliferation of naive peripheral CD4+ T cells in the absence of self peptide:MHC complexes. J Immunol *165*, 2458-2464.

Coates, N.J., and McColl, S.R. (2001). Production of chemokines in vivo in response to microbial stimulation. J Immunol *166*, 5176-5182.

Conlon, P.J., Morrissey, P.J., Nordan, R.P., Grabstein, K.H., Prickett, K.S., Reed, S.G., Goodwin, R., Cosman, D., and Namen, A.E. (1989). Murine thymocytes proliferate in direct response to interleukin-7. Blood *74*, 1368-1373.

Cook, J.R., Wormstall, E.M., Hornell, T., Russell, J., Connolly, J.M., and Hansen, T.H. (1997). Quantitation of the cell surface level of Ld resulting in positive versus negative selection of the 2C transgenic T cell receptor in vivo. Immunity *7*, 233-241.

Cory, S. (1995). Regulation of lymphocyte survival by the bcl-2 gene family. Annual review of immunology *13*, 513-543.

Cosgrove, D., Gray, D., Dierich, A., Kaufman, J., Lemeur, M., Benoist, C., and Mathis, D. (1991). Mice lacking MHC class II molecules. Cell *66*, 1051-1066.

Croft, M., Bradley, L.M., and Swain, S.L. (1994). Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. J Immunol *152*, 2675-2685.

Culty, M., Miyake, K., Kincade, P.W., Sikorski, E., Butcher, E.C., and Underhill, C. (1990). The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. The Journal of cell biology *111*, 2765-2774.

Curtsinger, J.M., Lins, D.C., and Mescher, M.F. (1998). CD8+ memory T cells (CD44high, Ly-6C+) are more sensitive than naive cells to (CD44low, Ly-6C-) to TCR/CD8 signaling in response to antigen. J Immunol *160*, 3236-3243.

Curtsinger, J.M., Schmidt, C.S., Mondino, A., Lins, D.C., Kedl, R.M., Jenkins, M.K., and Mescher, M.F. (1999). Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. J Immunol *162*, 3256-3262.

Curtsinger, J.M., Valenzuela, J.O., Agarwal, P., Lins, D., and Mescher, M.F. (2005). Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. J Immunol *174*, 4465-4469.

D

D'Souza, W.N., and Hedrick, S.M. (2006). Cutting edge: latecomer CD8 T cells are imprinted with a unique differentiation program. J Immunol *177*, 777-781.

De Magistris, M.T., Alexander, J., Coggeshall, M., Altman, A., Gaeta, F.C., Grey, H.M., and Sette, A. (1992). Antigen analog-major histocompatibility complexes act as antagonists of the T cell receptor. Cell *68*, 625-634.

Donskoy, E., and Goldschneider, I. (1992). Thymocytopoiesis is maintained by blood-borne precursors throughout postnatal life. A study in parabiotic mice. J Immunol *148*, 1604-1612.

Dorfman, J.R., Stefanova, I., Yasutomo, K., and Germain, R.N. (2000). CD4+ T cell survival is not directly linked to self-MHC-induced TCR signaling. Nature immunology *1*, 329-335.

Dutton, R.W., Bradley, L.M., and Swain, S.L. (1998). T cell memory. Annual review of immunology *16*, 201-223.

E

Ernst, B., Lee, D.S., Chang, J.M., Sprent, J., and Surh, C.D. (1999). The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. Immunity *11*, 173-181.

F

Fas, S.C., Fritzsching, B., Suri-Payer, E., and Krammer, P.H. (2006). Death receptor signaling and its function in the immune system. Current directions in autoimmunity *9*, 1-17.

Fehniger, T.A., Suzuki, K., Ponnappan, A., VanDeusen, J.B., Cooper, M.A., Florea, S.M., Freud, A.G., Robinson, M.L., Durbin, J., and Caligiuri, M.A. (2001). Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. The Journal of experimental medicine *193*, 219-231.

Fierer, J., Swancutt, M.A., Heumann, D., and Golenbock, D. (2002). The role of lipopolysaccharide binding protein in resistance to Salmonella infections in mice. J Immunol *168*, 6396-6403.

Fink, S.L., and Cookson, B.T. (2007). Pyroptosis and host cell death responses during Salmonella infection. Cellular microbiology *9*, 2562-2570.

Foulds, K.E., Zenewicz, L.A., Shedlock, D.J., Jiang, J., Troy, A.E., and Shen, H. (2002). Cutting edge: CD4 and CD8 T cells are intrinsically different in their proliferative responses. J Immunol *168*, 1528-1532.

Freitas, A.A., Agenes, F., and Coutinho, G.C. (1996). Cellular competition modulates survival and selection of CD8+ T cells. European journal of immunology *26*, 2640-2649.

Freitas, A.A., and Rocha, B. (2000). Population biology of lymphocytes: the flight for survival. Annual review of immunology *18*, 83-111.

Freitas, A.A., Rocha, B., and Coutinho, A.A. (1986). Lymphocyte population kinetics in the mouse. Immunological reviews *91*, 5-37.

Freitas, A.A., and Rocha, B.B. (1993). Lymphocyte lifespans: homeostasis, selection and competition. Immunology today *14*, 25-29.

Fukui, Y., Ishimoto, T., Utsuyama, M., Gyotoku, T., Koga, T., Nakao, K., Hirokawa, K., Katsuki, M., and Sasazuki, T. (1997). Positive and negative CD4+ thymocyte selection by a single MHC class II/peptide ligand affected by its expression level in the thymus. Immunity *6*, 401-410.

G

Garcia, S., DiSanto, J., and Stockinger, B. (1999). Following the development of a CD4 T cell response in vivo: from activation to memory formation. Immunity *11*, 163-171.

Ge, Q., Bai, A., Jones, B., Eisen, H.N., and Chen, J. (2004). Competition for self-peptide-MHC complexes and cytokines between naive and memory CD8+ T cells expressing the same or different T cell receptors. Proceedings of the National Academy of Sciences of the United States of America *101*, 3041-3046.

Germain, R.N. (1994). MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell *76*, 287-299.

Godfrey, D.I., Kennedy, J., Mombaerts, P., Tonegawa, S., and Zlotnik, A. (1994). Onset of TCR-beta gene rearrangement and role of TCR-beta expression during CD3-CD4-CD8- thymocyte differentiation. J Immunol *152*, 4783-4792.

Godfrey, D.I., Kennedy, J., Suda, T., and Zlotnik, A. (1993). A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J Immunol *150*, 4244-4252.

Godfrey, D.I., and Zlotnik, A. (1993). Control points in early T-cell development. Immunology today *14*, 547-553.

Goldrath, A.W., and Bevan, M.J. (1999). Low-affinity ligands for the TCR drive proliferation of mature CD8+ T cells in lymphopenic hosts. Immunity *11*, 183-190.

Goldrath, A.W., Luckey, C.J., Park, R., Benoist, C., and Mathis, D. (2004). The molecular program induced in T cells undergoing homeostatic proliferation. Proceedings of the National Academy of Sciences of the United States of America *101*, 16885-16890.

Goldrath, A.W., Sivakumar, P.V., Glaccum, M., Kennedy, M.K., Bevan, M.J., Benoist, C., Mathis, D., and Butz, E.A. (2002). Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory CD8+ T cells. The Journal of experimental medicine *195*, 1515-1522.

Grabstein, K.H., Eisenman, J., Shanebeck, K., Rauch, C., Srinivasan, S., Fung, V., Beers, C., Richardson, J., Schoenborn, M.A., Ahdieh, M., and et al. (1994). Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science (New York, N.Y *264*, 965-968.

Grabstein, K.H., Waldschmidt, T.J., Finkelman, F.D., Hess, B.W., Alpert, A.R., Boiani, N.E., Namen, A.E., and Morrissey, P.J. (1993). Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. The Journal of experimental medicine *178*, 257-264.

Grandjean, I., Duban, L., Bonney, E.A., Corcuff, E., Di Santo, J.P., Matzinger, P., and Lantz, O. (2003). Are major histocompatibility complex molecules involved in the survival of naive CD4+ T cells? The Journal of experimental medicine *198*, 1089-1102.

Gray, D., and Matzinger, P. (1991). T cell memory is short-lived in the absence of antigen. The Journal of experimental medicine *174*, 969-974.

Greenwald, R.J., Freeman, G.J., and Sharpe, A.H. (2005). The B7 family revisited. Annual review of immunology *23*, 515-548.

Gresser, I., Guy-Grand, D., Maury, C., and Maunoury, M.T. (1981). Interferon induces peripheral lymphadenopathy in mice. J Immunol *127*, 1569-1575.

Grusby, M.J., Johnson, R.S., Papaioannou, V.E., and Glimcher, L.H. (1991). Depletion of CD4+ T cells in major histocompatibility complex class II-deficient mice. Science (New York, N.Y *253*, 1417-1420.

H

Hagen, K.A., Moses, C.T., Drasler, E.F., Podetz-Pedersen, K.M., Jameson, S.C., and Khoruts, A. (2004). A role for CD28 in lymphopenia-induced proliferation of CD4 T cells. J Immunol *173*, 3909-3915.

Hand, T.W., Morre, M., and Kaech, S.M. (2007). Expression of IL-7 receptor alpha is necessary but not sufficient for the formation of memory CD8 T cells during viral infection. Proceedings of the National Academy of Sciences of the United States of America *104*, 11730-11735.

Hao, Y., Legrand, N., and Freitas, A.A. (2006). The clone size of peripheral CD8 T cells is regulated by TCR promiscuity. The Journal of experimental medicine *203*, 1643-1649.

Happel, K.I., Dubin, P.J., Zheng, M., Ghilardi, N., Lockhart, C., Quinton, L.J., Odden, A.R., Shellito, J.E., Bagby, G.J., Nelson, S., and Kolls, J.K. (2005). Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. The Journal of experimental medicine *202*, 761-769.

Happel, K.I., Zheng, M., Young, E., Quinton, L.J., Lockhart, E., Ramsay, A.J., Shellito, J.E., Schurr, J.R., Bagby, G.J., Nelson, S., and Kolls, J.K. (2003). Cutting edge: roles of Toll-like receptor 4 and IL-23 in IL-17 expression in response to Klebsiella pneumoniae infection. J Immunol *170*, 4432-4436.

Haring, J.S., Badovinac, V.P., and Harty, J.T. (2006). Inflaming the CD8+ T cell response. Immunity *25*, 19-29.

Haring, J.S., Jing, X., Bollenbacher-Reilley, J., Xue, H.H., Leonard, W.J., and Harty, J.T. (2008). Constitutive Expression of IL-7 Receptor {alpha} Does Not Support Increased Expansion or Prevent Contraction of Antigen-Specific CD4 or CD8 T Cells following Listeria monocytogenes Infection. J Immunol *180*, 2855-2862.

Harrington, L.E., Janowski, K.M., Oliver, J.R., Zajac, A.J., and Weaver, C.T. (2008). Memory CD4 T cells emerge from effector T-cell progenitors. Nature *452*, 356-360.

Harty, J.T., and Badovinac, V.P. (2008). Shaping and reshaping CD8+ T-cell memory. Nature reviews *8*, 107-119.

Hassan, J.O., and Curtiss, R., 3rd (1994). Virulent Salmonella typhimurium-induced lymphocyte depletion and immunosuppression in chickens. Infection and immunity *62*, 2027-2036.

He, Y.W., and Malek, T.R. (1998). The structure and function of gamma c-dependent cytokines and receptors: regulation of T lymphocyte development and homeostasis. Critical reviews in immunology *18*, 503-524.

Henry, T., Brotcke, A., Weiss, D.S., Thompson, L.J., and Monack, D.M. (2007). Type I interferon signaling is required for activation of the inflammasome during Francisella infection. The Journal of experimental medicine *204*, 987-994.

Hess, J., Ladel, C., Miko, D., and Kaufmann, S.H. (1996). Salmonella typhimurium aroA- infection in gene-targeted immunodeficient mice: major role of CD4+ TCR-alpha beta cells and IFN-gamma in bacterial clearance independent of intracellular location. J Immunol *156*, 3321-3326.

Hildeman, D.A., Zhu, Y., Mitchell, T.C., Bouillet, P., Strasser, A., Kappler, J., and Marrack, P. (2002). Activated T cell death in vivo mediated by proapoptotic bcl-2 family member bim. Immunity *16*, 759-767.

Hogquist, K.A., Jameson, S.C., Heath, W.R., Howard, J.L., Bevan, M.J., and Carbone, F.R. (1994). T cell receptor antagonist peptides induce positive selection. Cell *76*, 17-27.

Homann, D., Teyton, L., and Oldstone, M.B. (2001). Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. Nature medicine *7*, 913-919.

Hormaeche, C.E., Mastroeni, P., Harrison, J.A., Demarco de Hormaeche, R., Svenson, S., and Stocker, B.A. (1996). Protection against oral challenge three months after i.v. immunization of BALB/c mice with live Aro Salmonella typhimurium and Salmonella enteritidis vaccines is serotype (species)-dependent and only partially determined by the main LPS O antigen. Vaccine *14*, 251-259.

Huang, W., Na, L., Fidel, P.L., and Schwarzenberger, P. (2004). Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. The Journal of infectious diseases *190*, 624-631.

Hughes, E.N., Mengod, G., and August, J.T. (1981). Murine cell surface glycoproteins. Characterization of a major component of 80,000 daltons as a polymorphic differentiation antigen of mesenchymal cells. The Journal of biological chemistry *256*, 7023-7027.

Hughes, P.D., Belz, G.T., Fortner, K.A., Budd, R.C., Strasser, A., and Bouillet, P. (2008). Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. Immunity *28*, 197-205.

Huster, K.M., Koffler, M., Stemberger, C., Schiemann, M., Wagner, H., and Busch, D.H. (2006). Unidirectional development of CD8+ central memory T cells into protective Listeria-specific effector memory T cells. European journal of immunology *36*, 1453-1464.

I

Ignatowicz, L., Kappler, J., Parker, D.C., and Marrack, P. (1996). The responses of mature T cells are not necessarily antagonized by their positively selecting peptide. J Immunol *157*, 1827-1831.

Ivanova, N.B., Dimos, J.T., Schaniel, C., Hackney, J.A., Moore, K.A., and Lemischka, I.R. (2002). A stem cell molecular signature. Science (New York, N.Y *298*, 601-604.

J

Jacob, J., and Baltimore, D. (1999). Modelling T-cell memory by genetic marking of memory T cells in vivo. Nature *399*, 593-597.

Jameson, S.C. (2002). Maintaining the norm: T-cell homeostasis. Nature reviews *2*, 547-556.

Jameson, S.C., Hogquist, K.A., and Bevan, M.J. (1994). Specificity and flexibility in thymic selection. Nature *369*, 750-752.

Jameson, S.C., Hogquist, K.A., and Bevan, M.J. (1995). Positive selection of thymocytes. Annual review of immunology *13*, 93-126.

Janeway, C.A., Jr. (1992). High fives or hand clasps? Curr Biol *2*, 591-593.

Janssen, E.M., Droin, N.M., Lemmens, E.E., Pinkoski, M.J., Bensinger, S.J., Ehst, B.D., Griffith, T.S., Green, D.R., and Schoenberger, S.P. (2005). CD4+ T-cell help controls CD8+ T-cell memory via TRAIL-mediated activation-induced cell death. Nature *434*, 88-93.

Jelley-Gibbs, D.M., Brown, D.M., Dibble, J.P., Haynes, L., Eaton, S.M., and Swain, S.L. (2005). Unexpected prolonged presentation of influenza antigens promotes CD4 T cell memory generation. The Journal of experimental medicine *202*, 697-706.

Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D., Mascarenhas, M.M., Garg, H.G., Quinn, D.A., *et al.* (2005a). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nature medicine *11*, 1173-1179.

Jiang, H.Q., Kushnir, N., Thurnheer, M.C., Bos, N.A., and Cebra, J.J. (2002). Monoassociation of SCID mice with Helicobacter muridarum, but not four other enterics, provokes IBD upon receipt of T cells. Gastroenterology *122*, 1346-1354.

Jiang, J., Anaraki, F., Blank, K.J., and Murasko, D.M. (2003a). Cuttine edge: T cells from aged mice are resistant to depletion early during virus infection. J Immunol *171*, 3353-3357.

Jiang, J., Gross, D., Nogusa, S., Elbaum, P., and Murasko, D.M. (2005b). Depletion of T cells by type I interferon: differences between young and aged mice. J Immunol *175*, 1820-1826.

Jiang, J., Lau, L.L., and Shen, H. (2003b). Selective depletion of nonspecific T cells during the early stage of immune responses to infection. J Immunol *171*, 4352-4358.

Joshi, N.S., Cui, W., Chandele, A., Lee, H.K., Urso, D.R., Hagman, J., Gapin, L., and Kaech, S.M. (2007). Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. Immunity *27*, 281-295.

Judge, A.D., Zhang, X., Fujii, H., Surh, C.D., and Sprent, J. (2002). Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8(+) T cells. The Journal of experimental medicine *196*, 935-946.

K

Kaech, S.M., and Ahmed, R. (2001). Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. Nature immunology *2*, 415-422.

Kaech, S.M., Hemby, S., Kersh, E., and Ahmed, R. (2002). Molecular and functional profiling of memory CD8 T cell differentiation. Cell *111*, 837-851.

Kaech, S.M., Tan, J.T., Wherry, E.J., Konieczny, B.T., Surh, C.D., and Ahmed, R. (2003). Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nature immunology *4*, 1191-1198.

Kalinski, P., and Moser, M. (2005). Consensual immunity: success-driven development of T-helper-1 and T-helper-2 responses. Nature reviews *5*, 251-260.

Kallies, A., Hawkins, E.D., Belz, G.T., Metcalf, D., Hommel, M., Corcoran, L.M., Hodgkin, P.D., and Nutt, S.L. (2006). Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. Nature immunology *7*, 466-474.

Kamimura, D., and Bevan, M.J. (2007). Naive CD8+ T cells differentiate into protective memory-like cells after IL-2 anti IL-2 complex treatment in vivo. The Journal of experimental medicine *204*, 1803-1812.

Kamphuis, E., Junt, T., Waibler, Z., Forster, R., and Kalinke, U. (2006). Type I interferons directly regulate lymphocyte recirculation and cause transient blood lymphopenia. Blood *108*, 3253-3261.

Kassiotis, G., Garcia, S., Simpson, E., and Stockinger, B. (2002). Impairment of immunological memory in the absence of MHC despite survival of memory T cells. Nature immunology *3*, 244-250.

Kassiotis, G., Zamoyska, R., and Stockinger, B. (2003). Involvement of avidity for major histocompatibility complex in homeostasis of naive and memory T cells. The Journal of experimental medicine *197*, 1007-1016.

Kawana, H., Karaki, H., Higashi, M., Miyazaki, M., Hilberg, F., Kitagawa, M., and Harigaya, K. (2008). CD44 suppresses TLR-mediated inflammation. J Immunol *180*, 4235-4245.

Kaye, J., Hsu, M.L., Sauron, M.E., Jameson, S.C., Gascoigne, N.R., and Hedrick, S.M. (1989). Selective development of CD4+ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor. Nature *341*, 746-749.

Kedzierska, K., Stambas, J., Jenkins, M.R., Keating, R., Turner, S.J., and Doherty, P.C. (2007). Location rather than CD62L phenotype is critical in the early establishment of influenza-specific CD8+ T cell memory. Proceedings of the National Academy of Sciences of the United States of America *104*, 9782-9787.

Kennedy, M.K., Glaccum, M., Brown, S.N., Butz, E.A., Viney, J.L., Embers, M., Matsuki, N., Charrier, K., Sedger, L., Willis, C.R.*, et al.* (2000). Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. The Journal of experimental medicine *191*, 771-780.

Kieper, W.C., Burghardt, J.T., and Surh, C.D. (2004). A role for TCR affinity in regulating naive T cell homeostasis. J Immunol *172*, 40-44.

Kieper, W.C., Tan, J.T., Bondi-Boyd, B., Gapin, L., Sprent, J., Ceredig, R., and Surh, C.D. (2002). Overexpression of interleukin (IL)-7 leads to IL-15-independent generation of memory phenotype CD8+ T cells. The Journal of experimental medicine *195*, 1533-1539.

Kieper, W.C., Troy, A., Burghardt, J.T., Ramsey, C., Lee, J.Y., Jiang, H.Q., Dummer, W., Shen, H., Cebra, J.J., and Surh, C.D. (2005). Recent immune status determines the source of antigens that drive homeostatic T cell expansion. J Immunol *174*, 3158-3163.

Kim, K., Lee, C.K., Sayers, T.J., Muegge, K., and Durum, S.K. (1998). The trophic action of IL-7 on pro-T cells: inhibition of apoptosis of pro-T1, -T2, and -T3 cells correlates with Bcl-2 and Bax levels and is independent of Fas and p53 pathways. J Immunol *160*, 5735-5741.

Kim, S.K., Brehm, M.A., Welsh, R.M., and Selin, L.K. (2002). Dynamics of memory T cell proliferation under conditions of heterologous immunity and bystander stimulation. J Immunol *169*, 90-98.

Kim, S.K., Schluns, K.S., and Lefrancois, L. (1999). Induction and visualization of mucosal memory CD8 T cells following systemic virus infection. J Immunol *163*, 4125-4132.

Kim, S.K., and Welsh, R.M. (2004). Comprehensive early and lasting loss of memory CD8 T cells and functional memory during acute and persistent viral infections. J Immunol *172*, 3139-3150.

Kirberg, J., Berns, A., and von Boehmer, H. (1997). Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex-encoded molecules. The Journal of experimental medicine *186*, 1269-1275.

Kirby, A.C., Yrlid, U., Svensson, M., and Wick, M.J. (2001). Differential involvement of dendritic cell subsets during acute Salmonella infection. J Immunol *166*, 6802-6811.

Kirby, A.C., Yrlid, U., and Wick, M.J. (2002). The innate immune response differs in primary and secondary Salmonella infection. J Immunol *169*, 4450-4459.

Koller, B.H., Marrack, P., Kappler, J.W., and Smithies, O. (1990). Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. Science (New York, N.Y *248*, 1227-1230.

Kolumam, G.A., Thomas, S., Thompson, L.J., Sprent, J., and Murali-Krishna, K. (2005). Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. The Journal of experimental medicine *202*, 637-650.

Kondrack, R.M., Harbertson, J., Tan, J.T., McBreen, M.E., Surh, C.D., and Bradley, L.M. (2003). Interleukin 7 regulates the survival and generation of memory CD4 cells. The Journal of experimental medicine *198*, 1797-1806.

Korsmeyer, S.J. (1995). Regulators of cell death. Trends Genet *11*, 101-105.

L

La Gruta, N.L., Driel, I.R., and Gleeson, P.A. (2000). Peripheral T cell expansion in lymphopenic mice results in a restricted T cell repertoire. European journal of immunology *30*, 3380-3386.

Labrecque, N., Whitfield, L.S., Obst, R., Waltzinger, C., Benoist, C., and Mathis, D. (2001). How much TCR does a T cell need? Immunity *15*, 71-82.

Lacombe, M.H., Hardy, M.P., Rooney, J., and Labrecque, N. (2005). IL-7 receptor expression levels do not identify CD8+ memory T lymphocyte precursors following peptide immunization. J Immunol *175*, 4400-4407.

Lantz, O., Grandjean, I., Matzinger, P., and Di Santo, J.P. (2000). Gamma chain required for naive CD4+ T cell survival but not for antigen proliferation. Nature immunology *1*, 54-58.

Lanzavecchia, A., and Sallusto, F. (2002). Progressive differentiation and selection of the fittest in the immune response. Nature reviews *2*, 982-987.

Lauvau, G., Vijh, S., Kong, P., Horng, T., Kerksiek, K., Serbina, N., Tuma, R.A., and Pamer, E.G. (2001). Priming of memory but not effector CD8 T cells by a killed bacterial vaccine. Science (New York, N.Y *294*, 1735-1739.

Lenschow, D.J., Walunas, T.L., and Bluestone, J.A. (1996). CD28/B7 system of T cell costimulation. Annual review of immunology *14*, 233-258.

Lenz, D.C., Kurz, S.K., Lemmens, E., Schoenberger, S.P., Sprent, J., Oldstone, M.B., and Homann, D. (2004). IL-7 regulates basal homeostatic proliferation of antiviral CD4+T cell memory. Proceedings of the National Academy of Sciences of the United States of America *101*, 9357-9362.

Li, H., Zhu, H., Xu, C.J., and Yuan, J. (1998). Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell *94*, 491-501.

Li, J., Huston, G., and Swain, S.L. (2003). IL-7 promotes the transition of CD4 effectors to persistent memory cells. The Journal of experimental medicine *198*, 1807-1815.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S., and Wang, X. (1997). Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell *91*, 479-489.

Lin, J.X., Migone, T.S., Tsang, M., Friedmann, M., Weatherbee, J.A., Zhou, L., Yamauchi, A., Bloom, E.T., Mietz, J., John, S., and et al. (1995). The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity *2*, 331-339.

Lin, S.J., Chen, A.T., and Welsh, R.M. (2008). An immune system derived from homeostatic proliferation generates normal CD8 T cell memory but altered repertoires and diminished heterologous immune responses. Blood.

Liu, N., Phillips, T., Zhang, M., Wang, Y., Opferman, J.T., Shah, R., and Ashton-Rickardt, P.G. (2004). Serine protease inhibitor 2A is a protective factor for memory T cell development. Nature immunology *5*, 919-926.

Lo, W.F., Ong, H., Metcalf, E.S., and Soloski, M.J. (1999). T cell responses to Gram-negative intracellular bacterial pathogens: a role for CD8+ T cells in immunity to Salmonella infection and the involvement of MHC class Ib molecules. J Immunol *162*, 5398-5406.

Lodolce, J.P., Boone, D.L., Chai, S., Swain, R.E., Dassopoulos, T., Trettin, S., and Ma, A. (1998). IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. Immunity *9*, 669-676.

London, C.A., Lodge, M.P., and Abbas, A.K. (2000). Functional responses and costimulator dependence of memory CD4+ T cells. J Immunol *164*, 265-272.

Luckey, C.J., Bhattacharya, D., Goldrath, A.W., Weissman, I.L., Benoist, C., and Mathis, D. (2006). Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America *103*, 3304-3309.

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998). Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell *94*, 481-490.

Luu, R.A., Gurnani, K., Dudani, R., Kammara, R., van Faassen, H., Sirard, J.C., Krishnan, L., and Sad, S. (2006). Delayed expansion and contraction of CD8+ T cell response during infection with virulent Salmonella typhimurium. J Immunol *177*, 1516-1525.

M

Mackall, C.L., Bare, C.V., Granger, L.A., Sharrow, S.O., Titus, J.A., and Gress, R.E. (1996). Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol *156*, 4609-4616.

Mackall, C.L., Hakim, F.T., and Gress, R.E. (1997). Restoration of T-cell homeostasis after T-cell depletion. Seminars in immunology *9*, 339-346.

Mackey, M.F., Gunn, J.R., Maliszewsky, C., Kikutani, H., Noelle, R.J., and Barth, R.J., Jr. (1998). Dendritic cells require maturation via CD40 to generate protective antitumor immunity. J Immunol *161*, 2094-2098.
Madakamutil, L.T., Christen, U., Lena, C.J., Wang-Zhu, Y., Attinger, A., Sundarrajan, M., Ellmeier, W., von Herrath, M.G., Jensen, P., Littman, D.R., and Cheroutre, H. (2004). CD8alphaalpha-mediated survival and differentiation of CD8 memory T cell precursors. Science (New York, N.Y *304*, 590-593.

Maini, M.K., Gudgeon, N., Wedderburn, L.R., Rickinson, A.B., and Beverley, P.C. (2000). Clonal expansions in acute EBV infection are detectable in the CD8 and not the CD4 subset and persist with a variable CD45 phenotype. J Immunol *165*, 5729-5737.

Mannie, M.D. (1991). A unified model for T cell antigen recognition and thymic selection of the T cell repertoire. Journal of theoretical biology *151*, 169-192.

Margolick, J.B., and Donnenberg, A.D. (1997). T-cell homeostasis in HIV-1 infection. Seminars in immunology *9*, 381-388.

Markiewicz, M.A., Girao, C., Opferman, J.T., Sun, J., Hu, Q., Agulnik, A.A., Bishop, C.E., Thompson, C.B., and Ashton-Rickardt, P.G. (1998). Long-term T cell memory requires the surface expression of self-peptide/major histocompatibility complex molecules. Proceedings of the National Academy of Sciences of the United States of America *95*, 3065-3070.

Martin, B., Bourgeois, C., Dautigny, N., and Lucas, B. (2003). On the role of MHC class II molecules in the survival and lymphopenia-induced proliferation of peripheral CD4+ T cells. Proceedings of the National Academy of Sciences of the United States of America *100*, 6021-6026.

Martins, G.A., Cimmino, L., Shapiro-Shelef, M., Szabolcs, M., Herron, A., Magnusdottir, E., and Calame, K. (2006). Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nature immunology *7*, 457-465.

Marzo, A.L., Klonowski, K.D., Le Bon, A., Borrow, P., Tough, D.F., and Lefrancois, L. (2005). Initial T cell frequency dictates memory CD8+ T cell lineage commitment. Nature immunology *6*, 793-799.

Masopust, D., Vezys, V., Marzo, A.L., and Lefrancois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. Science (New York, N.Y *291*, 2413-2417.

Mastroeni, P., Villarreal-Ramos, B., and Hormaeche, C.E. (1992). Role of T cells, TNF alpha and IFN gamma in recall of immunity to oral challenge with virulent salmonellae in mice vaccinated with live attenuated aro- Salmonella vaccines. Microbial pathogenesis *13*, 477-491.

McNally, J.M., Zarozinski, C.C., Lin, M.Y., Brehm, M.A., Chen, H.D., and Welsh, R.M. (2001). Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. Journal of virology *75*, 5965-5976.

McSorley, S.J., Asch, S., Costalonga, M., Reinhardt, R.L., and Jenkins, M.K. (2002). Tracking salmonella-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. Immunity *16*, 365-377.

Mercado, R., Vijh, S., Allen, S.E., Kerksiek, K., Pilip, I.M., and Pamer, E.G. (2000). Early programming of T cell populations responding to bacterial infection. J Immunol *165*, 6833-6839.

Miller, R.A., and Stutman, O. (1984). T cell repopulation from functionally restricted splenic progenitors: 10,000-fold expansion documented by using limiting dilution analyses. J Immunol *133*, 2925-2932.

Min, B., Foucras, G., Meier-Schellersheim, M., and Paul, W.E. (2004). Spontaneous proliferation, a response of naive CD4 T cells determined by the diversity of the memory cell repertoire. Proceedings of the National Academy of Sciences of the United States of America *101*, 3874-3879.

Min, B., Yamane, H., Hu-Li, J., and Paul, W.E. (2005). Spontaneous and homeostatic proliferation of CD4 T cells are regulated by different mechanisms. J Immunol *174*, 6039-6044.

Mittrucker, H.W., and Kaufmann, S.H. (2000). Immune response to infection with Salmonella typhimurium in mice. Journal of leukocyte biology *67*, 457-463.

Miyake, K., Underhill, C.B., Lesley, J., and Kincade, P.W. (1990). Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. The Journal of experimental medicine *172*, 69-75.

Monack, D.M., Detweiler, C.S., and Falkow, S. (2001). Salmonella pathogenicity island 2-dependent macrophage death is mediated in part by the host cysteine protease caspase-1. Cellular microbiology *3*, 825-837.

Morrison, L.A., Lukacher, A.E., Braciale, V.L., Fan, D.P., and Braciale, T.J. (1986). Differences in antigen presentation to MHC class I-and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. The Journal of experimental medicine *163*, 903-921.

Moses, C.T., Thorstenson, K.M., Jameson, S.C., and Khoruts, A. (2003). Competition for self ligands restrains homeostatic proliferation of naive CD4 T cells. Proceedings of the National Academy of Sciences of the United States of America *100*, 1185-1190.

Moulton, V.R., Bushar, N.D., Leeser, D.B., Patke, D.S., and Farber, D.L. (2006). Divergent generation of heterogeneous memory CD4 T cells. J Immunol *177*, 869-876.

Moulton, V.R., and Farber, D.L. (2006). Committed to memory: lineage choices for activated T cells. Trends in immunology *27*, 261-267.

Muller, U., Steinhoff, U., Reis, L.F., Hemmi, S., Pavlovic, J., Zinkernagel, R.M., and Aguet, M. (1994). Functional role of type I and type II interferons in antiviral defense. Science (New York, N.Y *264*, 1918-1921.

Murali-Krishna, K., and Ahmed, R. (2000). Cutting edge: naive T cells masquerading as memory cells. J Immunol *165*, 1733-1737.

Murali-Krishna, K., Altman, J.D., Suresh, M., Sourdive, D.J., Zajac, A.J., Miller, J.D., Slansky, J., and Ahmed, R. (1998). Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity *8*, 177-187.

Murali-Krishna, K., Lau, L.L., Sambhara, S., Lemonnier, F., Altman, J., and Ahmed, R. (1999). Persistence of memory CD8 T cells in MHC class I-deficient mice. Science (New York, N.Y *286*, 1377-1381.

Murphy, K.M., and Reiner, S.L. (2002). The lineage decisions of helper T cells. Nature reviews *2*, 933-944.

Muzio, M., Chinnaiyan, A.M., Kischkel, F.C., O'Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J.D., Zhang, M., Gentz, R.*, et al.* (1996). FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. Cell *85*, 817-827.

N

Nauciel, C. (1990). Role of CD4+ T cells and T-independent mechanisms in acquired resistance to Salmonella typhimurium infection. J Immunol *145*, 1265-1269.

Nesic, D., and Vukmanovic, S. (1998). MHC class I is required for peripheral accumulation of CD8+ thymic emigrants. J Immunol *160*, 3705-3712.

Nikolic-Zugic, J., and Bevan, M.J. (1990). Role of self-peptides in positively selecting the T-cell repertoire. Nature *344*, 65-67.

Nossal, G.J. (1994). Negative selection of lymphocytes. Cell *76*, 229-239.

O

O'Connell, R.M., Saha, S.K., Vaidya, S.A., Bruhn, K.W., Miranda, G.A., Zarnegar, B., Perry, A.K., Nguyen, B.O., Lane, T.F., Taniguchi, T.*, et al.* (2004). Type I interferon production enhances susceptibility to Listeria monocytogenes infection. The Journal of experimental medicine *200*, 437-445.

Obar, J.J., Khanna, K.M., and Lefrancois, L. (2008). Endogenous naive CD8+ T cell precursor frequency regulates primary and memory responses to infection. Immunity *28*, 859-869.

Oehen, S., and Brduscha-Riem, K. (1999). Naive cytotoxic T lymphocytes spontaneously acquire effector function in lymphocytopenic recipients: A pitfall for T cell memory studies? European journal of immunology *29*, 608-614.

Oehen, S., Waldner, H., Kundig, T.M., Hengartner, H., and Zinkernagel, R.M. (1992). Antivirally protective cytotoxic T cell memory to lymphocytic choriomeningitis virus is governed by persisting antigen. The Journal of experimental medicine *176*, 1273-1281.

Okada, H., Kobune, F., Sato, T.A., Kohama, T., Takeuchi, Y., Abe, T., Takayama, N., Tsuchiya, T., and Tashiro, M. (2000). Extensive lymphopenia due to apoptosis of uninfected lymphocytes in acute measles patients. Archives of virology *145*, 905-920.

Oura, C.A., Powell, P.P., and Parkhouse, R.M. (1998). African swine fever: a disease characterized by apoptosis. The Journal of general virology *79 (Pt 6)*, 1427-1438.

P

Page, D.M., Alexander, J., Snoke, K., Appella, E., Sette, A., Hedrick, S.M., and Grey, H.M. (1994). Negative selection of CD4+ CD8+ thymocytes by T-cell receptor peptide antagonists. Proceedings of the National Academy of Sciences of the United States of America *91*, 4057-4061.

Peacock, C.D., Kim, S.K., and Welsh, R.M. (2003). Attrition of virus-specific memory CD8+ T cells during reconstitution of lymphopenic environments. J Immunol *171*, 655-663.

Peschon, J.J., Morrissey, P.J., Grabstein, K.H., Ramsdell, F.J., Maraskovsky, E., Gliniak, B.C., Park, L.S., Ziegler, S.F., Williams, D.E., Ware, C.B.*, et al.* (1994). Early lymphocyte expansion

is severely impaired in interleukin 7 receptor-deficient mice. The Journal of experimental medicine *180*, 1955-1960.

Petrie, H.T., Scollay, R., and Shortman, K. (1992). Commitment to the T cell receptor-alpha beta or -gamma delta lineages can occur just prior to the onset of CD4 and CD8 expression among immature thymocytes. European journal of immunology *22*, 2185-2188.

Pie, S., Truffa-Bachi, P., Pla, M., and Nauciel, C. (1997). Th1 response in Salmonella typhimurium-infected mice with a high or low rate of bacterial clearance. Infection and immunity *65*, 4509-4514.

Plant, J., and Glynn, A.A. (1979). Locating salmonella resistance gene on mouse chromosome 1. Clinical and experimental immunology *37*, 1-6.

Plum, J., De Smedt, M., and Leclercq, G. (1993). Exogenous IL-7 promotes the growth of CD3-CD4-CD8-CD44+CD25+/- precursor cells and blocks the differentiation pathway of TCR-alpha beta cells in fetal thymus organ culture. J Immunol *150*, 2706-2716.

Polic, B., Kunkel, D., Scheffold, A., and Rajewsky, K. (2001). How alpha beta T cells deal with induced TCR alpha ablation. Proceedings of the National Academy of Sciences of the United States of America *98*, 8744-8749.

Porter, B.O., and Malek, T.R. (1999). IL-2Rbeta/IL-7Ralpha doubly deficient mice recapitulate the thymic and intraepithelial lymphocyte (IEL) developmental defects of gammac-/- mice: roles for both IL-2 and IL-15 in CD8alphaalpha IEL development. J Immunol *163*, 5906-5912.

Prlic, M., Blazar, B.R., Khoruts, A., Zell, T., and Jameson, S.C. (2001). Homeostatic expansion occurs independently of costimulatory signals. J Immunol *167*, 5664-5668.

Pure, E., and Cuff, C.A. (2001). A crucial role for CD44 in inflammation. Trends in molecular medicine *7*, 213-221.

R

Rafi-Janajreh, A.Q., Chen, D., Schmits, R., Mak, T.W., Grayson, R.L., Sponenberg, D.P., Nagarkatti, M., and Nagarkatti, P.S. (1999). Evidence for the involvement of CD44 in endothelial cell injury and induction of vascular leak syndrome by IL-2. J Immunol *163*, 1619-1627.

Rath, H.C., Schultz, M., Freitag, R., Dieleman, L.A., Li, F., Linde, H.J., Scholmerich, J., and Sartor, R.B. (2001). Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. Infection and immunity *69*, 2277-2285.

Rathmell, J.C., Lindsten, T., Zong, W.X., Cinalli, R.M., and Thompson, C.B. (2002). Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis. Nature immunology *3*, 932-939.

Reed, D.S., Hensley, L.E., Geisbert, J.B., Jahrling, P.B., and Geisbert, T.W. (2004). Depletion of peripheral blood T lymphocytes and NK cells during the course of ebola hemorrhagic Fever in cynomolgus macaques. Viral immunology *17*, 390-400.

Reinhardt, R.L., Khoruts, A., Merica, R., Zell, T., and Jenkins, M.K. (2001). Visualizing the generation of memory CD4 T cells in the whole body. Nature *410*, 101-105.

Rocha, B., Dautigny, N., and Pereira, P. (1989). Peripheral T lymphocytes: expansion potential and homeostatic regulation of pool sizes and CD4/CD8 ratios in vivo. European journal of immunology *19*, 905-911.

Rocha, B., Freitas, A.A., and Coutinho, A.A. (1983). Population dynamics of T lymphocytes. Renewal rate and expansion in the peripheral lymphoid organs. J Immunol *131*, 2158-2164.

Rocha, B., Grandien, A., and Freitas, A.A. (1995). Anergy and exhaustion are independent mechanisms of peripheral T cell tolerance. The Journal of experimental medicine *181*, 993-1003.

Rocha, B.B. (1987). Population kinetics of precursors of IL 2-producing peripheral T lymphocytes: evidence for short life expectancy, continuous renewal, and post-thymic expansion. J Immunol *139*, 365-372.

Roman, E., Miller, E., Harmsen, A., Wiley, J., Von Andrian, U.H., Huston, G., and Swain, S.L. (2002). CD4 effector T cell subsets in the response to influenza: heterogeneity, migration, and function. The Journal of experimental medicine *196*, 957-968.

Rooke, R., Waltzinger, C., Benoist, C., and Mathis, D. (1997). Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. Immunity *7*, 123-134.

Russell, J.H., Rush, B., Weaver, C., and Wang, R. (1993). Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. Proceedings of the National Academy of Sciences of the United States of America *90*, 4409-4413.

S

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature *401*, 708-712.

Sandau, M.M., Winstead, C.J., and Jameson, S.C. (2007). IL-15 is required for sustained lymphopenia-driven proliferation and accumulation of CD8 T cells. J Immunol *179*, 120-125.

Schattner, A., Meshorer, A., and Wallach, D. (1983). Involvement of interferon in virus-induced lymphopenia. Cellular immunology *79*, 11-25.

Schlecht, G., Garcia, S., Escriou, N., Freitas, A.A., Leclerc, C., and Dadaglio, G. (2004). Murine plasmacytoid dendritic cells induce effector/memory CD8+ T-cell responses in vivo after viral stimulation. Blood *104*, 1808-1815.

Schluns, K.S., Kieper, W.C., Jameson, S.C., and Lefrancois, L. (2000). Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nature immunology *1*, 426-432.

Schluns, K.S., Williams, K., Ma, A., Zheng, X.X., and Lefrancois, L. (2002). Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. J Immunol *168*, 4827-4831.

Sebzda, E., Mariathasan, S., Ohteki, T., Jones, R., Bachmann, M.F., and Ohashi, P.S. (1999). Selection of the T cell repertoire. Annual review of immunology *17*, 829-874.

Sebzda, E., Wallace, V.A., Mayer, J., Yeung, R.S., Mak, T.W., and Ohashi, P.S. (1994). Positive and negative thymocyte selection induced by different concentrations of a single peptide. Science (New York, N.Y *263*, 1615-1618.

Seddon, B., Tomlinson, P., and Zamoyska, R. (2003). Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nature immunology *4*, 680-686.

Seddon, B., and Zamoyska, R. (2002). TCR signals mediated by Src family kinases are essential for the survival of naive T cells. J Immunol *169*, 2997-3005.

Selin, L.K., Cornberg, M., Brehm, M.A., Kim, S.K., Calcagno, C., Ghersi, D., Puzone, R., Celada, F., and Welsh, R.M. (2004). CD8 memory T cells: cross-reactivity and heterologous immunity. Seminars in immunology *16*, 335-347.

Selin, L.K., Lin, M.Y., Kraemer, K.A., Pardoll, D.M., Schneck, J.P., Varga, S.M., Santolucito, P.A., Pinto, A.K., and Welsh, R.M. (1999). Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. Immunity *11*, 733-742.

Selin, L.K., Vergilis, K., Welsh, R.M., and Nahill, S.R. (1996). Reduction of otherwise remarkably stable virus-specific cytotoxic T lymphocyte memory by heterologous viral infections. The Journal of experimental medicine *183*, 2489-2499.

Sha, W.C., Nelson, C.A., Newberry, R.D., Kranz, D.M., Russell, J.H., and Loh, D.Y. (1988). Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. Nature *335*, 271-274.

Sinha, K., Mastroeni, P., Harrison, J., de Hormaeche, R.D., and Hormaeche, C.E. (1997). Salmonella typhimurium aroA, htrA, and aroD htrA mutants cause progressive infections in athymic (nu/nu) BALB/c mice. Infection and immunity *65*, 1566-1569.

Smith, D.K., Dudani, R., Pedras-Vasconcelos, J.A., Chapdelaine, Y., van Faassen, H., and Sad, S. (2002). Cross-reactive antigen is required to prevent erosion of established T cell memory and tumor immunity: a heterologous bacterial model of attrition. J Immunol *169*, 1197-1206.

Spain, L.M., Jorgensen, J.L., Davis, M.M., and Berg, L.J. (1994). A peptide antigen antagonist prevents the differentiation of T cell receptor transgenic thymocytes. J Immunol *152*, 1709-1717.

Sprent, J., and Surh, C.D. (2001). Generation and maintenance of memory T cells. Current opinion in immunology *13*, 248-254.

Sprent, J., and Tough, D.F. (2001). T cell death and memory. Science (New York, N.Y *293*, 245-248.

Srinivasan, A., Foley, J., and McSorley, S.J. (2004). Massive number of antigen-specific CD4 T cells during vaccination with live attenuated Salmonella causes interclonal competition. J Immunol *172*, 6884-6893.

Stemberger, C., Huster, K.M., Koffler, M., Anderl, F., Schiemann, M., Wagner, H., and Busch, D.H. (2007). A single naive CD8+ T cell precursor can develop into diverse effector and memory subsets. Immunity *27*, 985-997.

Stockinger, B., and Veldhoen, M. (2007). Differentiation and function of Th17 T cells. Current opinion in immunology *19*, 281-286.

Stout, R.D., and Suttles, J. (1992). T cells bearing the CD44hi "memory" phenotype display characteristics of activated cells in G1 stage of cell cycle. Cellular immunology *141*, 433-443.

Strasser, A. (2005). The role of BH3-only proteins in the immune system. Nature reviews *5*, 189-200.

Strasser, A., Harris, A.W., and Cory, S. (1991). bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell *67*, 889-899.

Strasser, A., Harris, A.W., Huang, D.C., Krammer, P.H., and Cory, S. (1995). Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. The EMBO journal *14*, 6136-6147.

Suda, T., Murray, R., Guidos, C., and Zlotnik, A. (1990). Growth-promoting activity of IL-1 alpha, IL-6, and tumor necrosis factor-alpha in combination with IL-2, IL-4, or IL-7 on murine thymocytes. Differential effects on CD4/CD8 subsets and on CD3+/CD3- double-negative thymocytes. J Immunol *144*, 3039-3045.

Suda, T., and Zlotnik, A. (1991). IL-7 maintains the T cell precursor potential of CD3-CD4-CD8- thymocytes. J Immunol *146*, 3068-3073.

Summerfield, A., Knotig, S.M., and McCullough, K.C. (1998). Lymphocyte apoptosis during classical swine fever: implication of activation-induced cell death. Journal of virology *72*, 1853-1861.

Swain, S.L., Hu, H., and Huston, G. (1999). Class II-independent generation of CD4 memory T cells from effectors. Science (New York, N.Y *286*, 1381-1383.

T

Takeda, S., Rodewald, H.R., Arakawa, H., Bluethmann, H., and Shimizu, T. (1996). MHC class II molecules are not required for survival of newly generated CD4+ T cells, but affect their long-term life span. Immunity *5*, 217-228.

Tan, J.T., Dudl, E., LeRoy, E., Murray, R., Sprent, J., Weinberg, K.I., and Surh, C.D. (2001). IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proceedings of the National Academy of Sciences of the United States of America *98*, 8732-8737.

Tan, J.T., Ernst, B., Kieper, W.C., LeRoy, E., Sprent, J., and Surh, C.D. (2002). Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. The Journal of experimental medicine *195*, 1523-1532.

Tanchot, C., Lemonnier, F.A., Perarnau, B., Freitas, A.A., and Rocha, B. (1997). Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science (New York, N.Y *276*, 2057-2062.

Tanchot, C., and Rocha, B. (1997). Peripheral selection of T cell repertoires: the role of continuous thymus output. The Journal of experimental medicine *186*, 1099-1106.

Teder, P., Vandivier, R.W., Jiang, D., Liang, J., Cohn, L., Pure, E., Henson, P.M., and Noble, P.W. (2002). Resolution of lung inflammation by CD44. Science (New York, N.Y *296*, 155-158.

Teh, H.S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Bluthmann, H., and von Boehmer, H. (1988). Thymic major histocompatibility complex antigens and the alpha beta T-cell receptor determine the CD4/CD8 phenotype of T cells. Nature *335*, 229-233.

Tew, J.G., and Mandel, T.E. (1979). Prolonged antigen half-life in the lymphoid follicles of specifically immunized mice. Immunology *37*, 69-76.

Tough, D.F., Borrow, P., and Sprent, J. (1996). Induction of bystander T cell proliferation by viruses and type I interferon in vivo. Science (New York, N.Y *272*, 1947-1950.

Tough, D.F., and Sprent, J. (1994). Turnover of naive- and memory-phenotype T cells. The Journal of experimental medicine *179*, 1127-1135.

Trowbridge, I.S., Lesley, J., Schulte, R., Hyman, R., and Trotter, J. (1982). Biochemical characterization and cellular distribution of a polymorphic, murine cell-surface glycoprotein expressed on lymphoid tissues. Immunogenetics *15*, 299-312.

Troy, A.E., and Shen, H. (2003). Cutting edge: homeostatic proliferation of peripheral T lymphocytes is regulated by clonal competition. J Immunol *170*, 672-676.

Tumpey, T.M., Lu, X., Morken, T., Zaki, S.R., and Katz, J.M. (2000). Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A (H5N1) virus isolated from humans. Journal of virology *74*, 6105-6116.

U

Unsoeld, H., Krautwald, S., Voehringer, D., Kunzendorf, U., and Pircher, H. (2002). Cutting edge: CCR7+ and CCR7- memory T cells do not differ in immediate effector cell function. J Immunol *169*, 638-641.

V

van der Velden, A.W., Lindgren, S.W., Worley, M.J., and Heffron, F. (2000). Salmonella pathogenicity island 1-independent induction of apoptosis in infected macrophages by Salmonella enterica serotype typhimurium. Infection and immunity *68*, 5702-5709.

van Faassen, H., Saldanha, M., Gilbertson, D., Dudani, R., Krishnan, L., and Sad, S. (2005). Reducing the stimulation of CD8+ T cells during infection with intracellular bacteria promotes differentiation primarily into a central (CD62LhighCD44high) subset. J Immunol *174*, 5341-5350.

Van Kaer, L., Ashton-Rickardt, P.G., Ploegh, H.L., and Tonegawa, S. (1992). TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4-8+ T cells. Cell *71*, 1205-1214.

van Stipdonk, M.J., Lemmens, E.E., and Schoenberger, S.P. (2001). Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. Nature immunology *2*, 423-429.

Varga, S.M., Selin, L.K., and Welsh, R.M. (2001). Independent regulation of lymphocytic choriomeningitis virus-specific T cell memory pools: relative stability of CD4 memory under conditions of CD8 memory T cell loss. J Immunol *166*, 1554-1561.

Veiga-Fernandes, H., Walter, U., Bourgeois, C., McLean, A., and Rocha, B. (2000). Response of naive and memory CD8+ T cells to antigen stimulation in vivo. Nature immunology *1*, 47-53.

Vella, A., Teague, T.K., Ihle, J., Kappler, J., and Marrack, P. (1997). Interleukin 4 (IL-4) or IL-7 prevents the death of resting T cells: stat6 is probably not required for the effect of IL-4. The Journal of experimental medicine *186*, 325-330.

Vidal, S., Tremblay, M.L., Govoni, G., Gauthier, S., Sebastiani, G., Malo, D., Skamene, E., Olivier, M., Jothy, S., and Gros, P. (1995). The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. The Journal of experimental medicine *182*, 655-666.

Viola, A., Schroeder, S., Sakakibara, Y., and Lanzavecchia, A. (1999). T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science (New York, N.Y *283*, 680-682.

Vivien, L., Benoist, C., and Mathis, D. (2001). T lymphocytes need IL-7 but not IL-4 or IL-6 to survive in vivo. International immunology *13*, 763-768.

von Boehmer, H. (1990). Developmental biology of T cells in T cell-receptor transgenic mice. Annual review of immunology *8*, 531-556.

von Boehmer, H. (1994). Positive selection of lymphocytes. Cell *76*, 219-228.

von Freeden-Jeffry, U., Vieira, P., Lucian, L.A., McNeil, T., Burdach, S.E., and Murray, R. (1995). Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. The Journal of experimental medicine *181*, 1519-1526.

W

Wakim, L.M., Waithman, J., van Rooijen, N., Heath, W.R., and Carbone, F.R. (2008). Dendritic cell-induced memory T cell activation in nonlymphoid tissues. Science (New York, N.Y *319*, 198-202.

Wang, Q., Strong, J., and Killeen, N. (2001). Homeostatic competition among T cells revealed by conditional inactivation of the mouse Cd4 gene. The Journal of experimental medicine *194*, 1721-1730.

Wang, X., Xu, L., Wang, H., Zhan, Y., Pure, E., and Feuerstein, G.Z. (2002). CD44 deficiency in mice protects brain from cerebral ischemia injury. Journal of neurochemistry *83*, 1172-1179.

Watts, T.H. (2005). TNF/TNFR family members in costimulation of T cell responses. Annual review of immunology *23*, 23-68.

Welsh, R.M., and Selin, L.K. (2002). No one is naive: the significance of heterologous T-cell immunity. Nature reviews *2*, 417-426.

Wherry, E.J., Teichgraber, V., Becker, T.C., Masopust, D., Kaech, S.M., Antia, R., von Andrian, U.H., and Ahmed, R. (2003). Lineage relationship and protective immunity of memory CD8 T cell subsets. Nature immunology *4*, 225-234.

Whitmire, J.K., Tan, J.T., and Whitton, J.L. (2005). Interferon-gamma acts directly on CD8+ T cells to increase their abundance during virus infection. The Journal of experimental medicine *201*, 1053-1059.

Williams, K.M., Hakim, F.T., and Gress, R.E. (2007). T cell immune reconstitution following lymphodepletion. Seminars in immunology *19*, 318-330.

Williams, O., Tanaka, Y., Tarazona, R., and Kioussis, D. (1997). The agonist-antagonist balance in positive selection. Immunology today *18*, 121-126.

Witherden, D., van Oers, N., Waltzinger, C., Weiss, A., Benoist, C., and Mathis, D. (2000). Tetracycline-controllable selection of CD4(+) T cells: half-life and survival signals in the absence of major histocompatibility complex class II molecules. The Journal of experimental medicine *191*, 355-364.

Wittig, B.M., Johansson, B., Zoller, M., Schwarzler, C., and Gunthert, U. (2000). Abrogation of experimental colitis correlates with increased apoptosis in mice deficient for CD44 variant exon 7 (CD44v7). The Journal of experimental medicine *191*, 2053-2064.

Wong, P., and Pamer, E.G. (2001). Cutting edge: antigen-independent CD8 T cell proliferation. J Immunol *166*, 5864-5868.

Wrenshall, L.E., Stevens, E.T., Smith, D.R., and Miller, J.D. (2007). Maternal microchimerism leads to the presence of interleukin-2 in interleukin-2 knock out mice: implications for the role of interleukin-2 in thymic function. Cellular immunology *245*, 80-90.

Wu, C.Y., Kirman, J.R., Rotte, M.J., Davey, D.F., Perfetto, S.P., Rhee, E.G., Freidag, B.L., Hill, B.J., Douek, D.C., and Seder, R.A. (2002a). Distinct lineages of T(H)1 cells have differential capacities for memory cell generation in vivo. Nature immunology *3*, 852-858.

Wu, T.S., Lee, J.M., Lai, Y.G., Hsu, J.C., Tsai, C.Y., Lee, Y.H., and Liao, N.S. (2002b). Reduced expression of Bcl-2 in CD8+ T cells deficient in the IL-15 receptor alpha-chain. J Immunol *168*, 705-712.

Y

Yajima, T., Nishimura, H., Ishimitsu, R., Watase, T., Busch, D.H., Pamer, E.G., Kuwano, H., and Yoshikai, Y. (2002). Overexpression of IL-15 in vivo increases antigen-driven memory CD8+ T cells following a microbe exposure. J Immunol *168*, 1198-1203.

Yang, K.K., Dorner, B.G., Merkel, U., Ryffel, B., Schutt, C., Golenbock, D., Freeman, M.W., and Jack, R.S. (2002). Neutrophil influx in response to a peritoneal infection with Salmonella is

delayed in lipopolysaccharide-binding protein or CD14-deficient mice. J Immunol *169*, 4475-4480.

Yin, X.M., Wang, K., Gross, A., Zhao, Y., Zinkel, S., Klocke, B., Roth, K.A., and Korsmeyer, S.J. (1999). Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. Nature *400*, 886-891.

Yrlid, U., Svensson, M., Hakansson, A., Chambers, B.J., Ljunggren, H.G., and Wick, M.J. (2001). In vivo activation of dendritic cells and T cells during Salmonella enterica serovar Typhimurium infection. Infection and immunity *69*, 5726-5735.

Z

Zammit, D.J., Cauley, L.S., Pham, Q.M., and Lefrancois, L. (2005). Dendritic cells maximize the memory CD8 T cell response to infection. Immunity *22*, 561-570.

Zammit, D.J., Turner, D.L., Klonowski, K.D., Lefrancois, L., and Cauley, L.S. (2006). Residual antigen presentation after influenza virus infection affects CD8 T cell activation and migration. Immunity *24*, 439-449.

Zhang, X., Sun, S., Hwang, I., Tough, D.F., and Sprent, J. (1998). Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity *8*, 591-599.

Zhang, Y., Schlossman, S.F., Edwards, R.A., Ou, C.N., Gu, J., and Wu, M.X. (2002). Impaired apoptosis, extended duration of immune responses, and a lupus-like autoimmune disease in IEX-1-transgenic mice. Proceedings of the National Academy of Sciences of the United States of America *99*, 878-883.

Zijlstra, M., Bix, M., Simister, N.E., Loring, J.M., Raulet, D.H., and Jaenisch, R. (1990). Beta 2-microglobulin deficient mice lack CD4-8+ cytolytic T cells. Nature *344*, 742-746.

Zimmermann, C., Prevost-Blondel, A., Blaser, C., and Pircher, H. (1999). Kinetics of the response of naive and memory CD8 T cells to antigen: similarities and differences. European journal of immunology *29*, 284-290.

Zuniga-Pflucker, J.C., and Lenardo, M.J. (1996). Regulation of thymocyte development from immature progenitors. Current opinion in immunology *8*, 215-224.