Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript
Faculty of Biology and Medicine Publication

serval
serveur académique lausannois

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Immunotherapeutic targeting of LIGHT/LTβR/HVEM pathway fully recapitulates the reduced cytotoxic phenotype of LIGHT-deficient T cells

Maria-Luisa del Rio [a,*], Carlos Fernandez-Renedo [a], Olivier Chaloin [b], Stefanie Scheu [c], Klaus Pfeffer [c], Yasushi Shintani [d], Jose-Antonio Perez-Simon [e], Pascal Schneider [f], and Jose-Ignacio Rodriguez-Barbosa [a,*]

[a] *Transplantation Immunobiology Section, Institute of Biomedicine, University of Leon and Castilla and Leon Regional Transplantation Coordination, Leon University Hospital, Leon, Spain*

[b] *CNRS UPR 3572, IBMC, Immunopathologie et Chimie Thérapeutique, 15 rue René Descartes, Strasbourg, France*

[c] *Institute of Medical Microbiology and Hospital Hygiene, University of Duesseldorf, Universitaetsstr. 1, Geb. 22.21, Duesseldorf, D-40225 Germany*

[d] *Department of International Affairs, Japan Science and Technology Agency, K′s Gobancho 7, Gobancho Chiyoda-Ku, Tokyo, 102-0076, Japan*

[e] *Department of Hematology, University Hospital Virgen del Rocio / Institute of Biomedicine (IBIS / CSIC), Sevilla, Spain.*

[f] *Department of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland*

Keywords: HVEM (TNFRSF14, CD270); LIGHT (TNFSF14, CD258); LTβR (TNFRSF3), DcR3 (TNFRSF6b), co-stimulation; transplantation; alloreactivity; graft rejection; graft versus host disease, cytotoxicity.

To whom correspondence should be addressed:

[*] Dr. J. I. Rodríguez-Barbosa, DVM, Ph.D. Transplantation Immunobiology Section, Institute of Biomedicine, University of Leon, 24007-Leon, Spain, Tel.: +34-987-293079, Fax: +34-987-291998. Email: ignacio.barbosa@unileon.es.

[*] Dr. M. L. del Rio, DVM, Ph.D. Transplantation Immunobiology Section, Institute of Biomedicine, University of Leon and Castilla and Leon Regional Transplantation Coordination, Leon University Hospital, Leon, Spain. Email: mlriogon@saludcastillayleon.es

List of abbreviations

LIGHT: homologous to lymphotoxin, exhibits inducible expression and competes with HSV glycoprotein D for binding to herpesvirus entry mediator, a receptor expressed on T lymphocytes

mAb: monoclonal antibody

GvHR: Graft versus host reaction

CD: Cluster of differentiation

HSV: Herpesvirus

TNF: Tumor necrosis factor

TNFR: Tumor necrosis factor receptor

HVEM: Herpesvirus entry mediator

LTβR: Lymphotoxin beta receptor

CRD: Cysteine rich domain

APC: Antigen presenting cells

FDC: Follicular dendritic cell

DC: Dendritic cell

CTL: Cytotoxic T lymphocyte

NK: Natural killer

WT: Wild type

KO: Knock-out

mGFP: monster green fluorescent protein

eGFP: enhanced green fluorescent protein

APC: Allophycocyanin

PE: Phycoerythrin

MHC: Major Histocompatibility Complex

PMA: Phorbol myristate acetate

CFSE: Carboxyfluorescein succinimidyl ester

FF-LIGHT: Flag-Foldon-tagged soluble mouse LIGHT.

HVEM-Ig: HVEM.mIgG$_{2a}$.Fc: Herpesvirus entry mediator bound to mouse IgG$_{2a}$ Fc fragment.

LTβR-Ig: LTβR.huIgG$_1$.Fc: Lymphotoxin beta receptor bound to human IgG1 Fc fragment.

Abstract

TNF/TNFR superfamily members play essential roles in the development of the different phases of the immune response. Mouse LIGHT (TNFSF14) is a type II transmembrane protein with a C-terminus extracellular TNF homology domain (THD) that assembles in homotrimers and regulates the course of the immune responses by signaling through two receptors, the herpes virus entry mediator (HVEM, TNFSFR14) and the lymphotoxin β receptor (LTβR, TNFSFR3). LIGHT is a membrane bound protein transiently expressed on activated T cells, NK cells and immature dendritic cells (DC) that can be proteolytically cleaved by a metalloprotease and released to the extracellular milieu. The immunotherapeutic potential of LIGHT blockade was evaluated *in vivo*. Administration of an antagonist of LIGHT interaction with its receptors attenuated the course of graft versus host reaction and recapitulated the reduced cytotoxic activity of LIGHT-deficient T cells adoptively transferred into non-irradiated semiallogeneic recipients. The lack of LIGHT expression on donor T cells or blockade of LIGHT interaction with its receptors slowed down the rate of T cell proliferation and decreased the frequency of precursor alloreactive T cells, retarding T cell differentiation towards effector T cells. The blockade of LIGHT/LTβR/HVEM pathway was associated with delayed downregulation of IL-7Rα and delayed upregulation of ICOS expression on donor alloreactive CD8 T cells that are typical features of impaired T cell differentiation. These results expose the relevance of LIGHT/LTβR/HVEM interaction for the potential therapeutic control of the allogeneic immune responses mediated by alloreactive CD8 T cells that can contribute to prolong allograft survival.

Introduction

The TNF/TNFR superfamily members play essential roles in diverse immunological processes such as T cell activation, costimulation, clonal expansion and T cell differentiation toward effector T cells. [1, 2, 3] The therapeutic intervention with biologics antagonizing the TNF/TNFR interactions can theoretically influence CD4 and CD8 T cell activation, clonal expansion, survival and particularly the process of differentiation toward effector T cells. This converts these interacting pathways as susceptible targets for the modulation of T cell-mediated immune responses.[4, 5]

Mouse LIGHT (TNFSF14) is a type II transmembrane protein of 239 amino acids with a C-terminal extracellular TNF homology domain (THD) that assembles as homotrimers capable to interact with HVEM [6] and LTβR.[7, 8] There are two isoforms of mouse LIGHT produced by alternative splicing: an isoform without transmembrane domain that resides in the cytosol, and an isoform with a transmembrane domain that can be proteolytically processed at amino acid 84 to generate a soluble extracellular form of LIGHT. [6, 9] In humans, there is an additional binding partner of LIGHT, a soluble protein named DcR3/TR6 protein (TNFRF6B) that lacks of transmembrane domain.[10, 11]

Mouse LIGHT displays a pattern of expression mainly restricted to activated T cells, NK cells and bone marrow immature dendritic cells. [6,12] Both LIGHT receptors, HVEM and LTβR, are expressed on hematopoietic and non-hematopoietic stromal cells, although the latter is not expressed on lymphoid cells.[13, 14] Whereas LIGHT/HVEM main functional activity is a cosignaling pathway in T cells, LIGHT/LTβR interaction seems to be more relevant in regulating stromal/APC/T cell cross-talk.[15]

Preclinical studies in mouse models of disease are required to establish the proof of concept for the function of a target that permits to propel clinical trials in non-human

primates and humans in order to validate the potential use of new biologics for the treatment of immune-mediated diseases. However, this demands the development of high quality *in vivo* reagents to target the molecule of interest and its interactions. Our group has previously shown that antibody-mediated blockade of LIGHT/LTβR interaction with a partial antagonist antibody of LIGHT/LTβR interaction reduced short-term cytotoxic allogeneic responses, although did not fully recapitulate the impaired cytotoxic response observed in LIGHT-deficient T cells.[12]

Based on some evidences in the field of transplantation using soluble LIGHT receptors as fusion proteins, such as HVEM.Ig and LTβR.Ig [16,17,18] and from the phenotype of LIGHT-deficient mice, [19, 12, 17] we postulated that a complete blockade of LIGHT interaction with its receptors would contribute to achieve a more suitable pharmacological control of the allogeneic immune response. LIGHT blockade on T cells would impede its interaction with LTβR or HVEM on DC and therefore would hinder their maturation, [19,20, 21, 22] as well as would prevent T/T cell collaboration through LIGHT/HVEM interactions that would contribute to maintain T cell survival during T cell expansion and differentiation. [23, 24, 25, 26] To confirm this hypothesis, we characterized a set of anti-LIGHT antibodies raised in LIGHT-deficient mice and chose one of them that fully blocked the binding of soluble LTβR or HVEM to membrane LIGHT.

We demonstrated that efficient blockade of both HVEM/LIGHT and LTβR/LIGHT interactions attenuated the allogeneic immune response in a mouse model of graft versus host reaction and fully recapitulated the reduced cytotoxic phenotype of allogeneic LIGHT-deficient T cells. This study points to LIGHT as a suitable target for a better immunotherapeutic control of cytotoxic responses in transplantation.

Results

Clone 3D11 is a neutralizing anti-LIGHT antibody that efficiently blocks its receptor-binding site

To demonstrate the *in vivo* impact of LIGHT interaction with its receptors in the course of the allogeneic immune response, an antagonist mouse anti-mouse LIGHT monoclonal antibody (clone 3D11, mouse IgG$_{2b}$, k) was raised in LIGHT-deficient mice immunized with LIGHT-transduced cells.

The 3D11 antibody selectively bound NIH 3T3 cells expressing a GFP:LIGHT fusion protein, in which GFP was fused to the intracellular N-terminus of mouse LIGHT, but did not recognize control NIH 3T3 cells expressing GFP only (Figure 1A, upper panel). The binding specificity was confirmed by pre-incubation of clone 3D11 with recombinant FF-LIGHT, which blocked the ability of 3D11 to bind LIGHT-transduced cells (Figure 1A, lower panel).

We next evaluated whether the anti-LIGHT mAb would prevent the interaction of mouse LIGHT expressed on cells with its receptors HVEM and LTβR. The 3D11 antibody antagonized the binding of sLTβR.Ig and sHVEM.Ig to mouse LIGHT-transduced cells down to background staining levels, similar to those obtained with isotype-matched control immunoglobulins (Figure 1B). The binding of anti-LIGHT mAb to LIGHT transduced cells was inhibited by FF-LIGHT at close to stoichiometry ratio assuming a monomeric antibody mass of 150 kDa and two recognition sites per antibody and 67.5 kDa for the FF-LIGHT trimer and three epitopes per molecule (at EC 50, 0.625 μg of antibody neutralized 1 μg FF-LIGHT) (Figure 1C).

We also assessed the binding avidity of the Fc fragment of anti-mouse LIGHT antibody to the activating and inhibiting mouse FcγR by surface plasmon resonance. The avidity constant (K_D = Kd/Ka) for CD64 (FcγRI) and CD16.2 (FcγRIV) was 84 μM

and 9.9 μM, whereas for CD16 (FcγRIII) and CD32 (FcγRIIB) was 0.41 μM and 0.23 μM respectively (Figure 1C).

We conclude from these results that 3D11 recognizes specifically mouse LIGHT but also fully inhibits the interaction of LIGHT with its receptors (HVEM and LTβR).

LIGHT protein expression is transiently detected upon polyclonal activation of T cells

As it occurs for some other members of the TNF superfamily ligands such as CD40L or human LIGHT, its expression is only transient on activated T cells, [13, 27] and visualization of this transient expression *in vitro* requires the presence of the fluorochrome-labeled antibody throughout the course of activation. [12] In C57BL/6 splenocytes stimulated for 5 h with PMA plus ionomycin, LIGHT expression was specifically detected with anti-LIGHT (3D11) antibody (Figure 2A), in line with results obtained previously with another anti-mouse LIGHT antibody. [12] Mouse LIGHT was only detected on activated CD4 and CD8 T cells, but not on resting T lymphocytes (Figure 2A). As expected for a specific staining, pre-incubation of the antibody with a molar excess of Flag-Foldon LIGHT abolished the staining of activated CD4$^+$ and CD8$^+$ T cells, respectively (Figure 2B).

Antibody mediated blockade of the LIGHT/LTβR/HVEM pathway recapitulates the attenuated cytotoxic allogeneic response of LIGHT-deficient T cells.

LIGHT is required for lymph node hypertrophy in response to antigen immunization [28], for T cell differentiation towards effector T cells in the course of an allogeneic immune responses [29, 19] and for anti-tumor immunity. [18, 30] Due to the lack of well-characterized anti-LIGHT antibodies, the therapeutic potential of modulating LIGHT has remained

elusive because selective blockade of LIGHT cannot be properly achieved with soluble HVEM.Ig or LTβR.Ig fusion proteins that both bind several ligands.

With the novel anti-LIGHT antibody, we addressed in mice whether blockade of the LIGHT/LTβR/HVEM pathway could attenuate the course of graft versus host reaction in a semiallogeneic adoptive transfer model of alloreactivity. To that aim, CB6F1 (F1) recipient mice adoptively received either F1 splenocytes (control), or semiallogeneic B6 splenocytes (to induce graft versus host reaction), or LIGHT-deficient B6 splenocytes (to induce graft versus host reaction in the absence of LIGHT on transferred cells). Mice adoptively transferred with semiallogeneic B6 splenocytes were treated with 3D11 (mouse anti-mouse LIGHT, isotype IgG$_{2b}$) or with an isotype control mouse IgG$_{2b}$. Nineteen days after the adoptive transfer, at the peak of the acute phase of the disease, the absolute number of host cells residing in the thymus, bone marrow and spleen was evaluated as a readout measurement of graft versus host reaction severity. [31, 32] As expected, normal host cell numbers were found in F1 mice receiving F1 splenocytes, but almost all hematopoietic host cells were rejected 19 days after transfer of semiallogeneic B6 splenocytes. An equally efficient rejection of hematopoietic host cells was observed in the bone marrow, thymus (total and double-positive thymocytes) and spleen, *i.e.* in primary and secondary lymphoid tissues (Figure 3A-D). Transfer of LIGHT-deficient B6 splenocytes still lead to host cell rejection, but with reduced severity compared to wild type (p<0.005), and the same was true when endogenous LIGHT was blocked with 3D11 upon transfer of wild type B6 splenocytes into F1 recipients (Figure 3). No significant differences were found between the latter two conditions.

These results suggest that LIGHT blockade recapitulates the attenuated cytotoxic phenotype of LIGHT-deficient alloreactive T cells in F1 recipients, pointing to the

relevance of LIGHT for the control of cytotoxic responses during the course of the allogeneic immune responses.

Blockade of LIGHT impairs allogeneic T cell proliferation almost as efficiently as its genetic ablation

To understand why LIGHT inhibition reduced the severity of the allogeneic cytotoxic response, we monitored the proliferative capacity of CFSE-labeled donor alloreactive B6 splenocytes transferred into F1 recipient mice, in the presence or absence of genetic deficient LIGHT or immune therapeutic inhibition of LIGHT. Three days after the adoptive transfer of donor splenocytes, percentages of precursors (PF) and proliferation indexes (PI) were measured for CFSE-labeled donor CD4^{+} and CD8^{+} T cells. The PF and PI were reduced in the presence of the anti-LIGHT antagonist antibody compared to treatment with the control antibody (Figure 4). Moreover, the reduction achieved with the anti-LIGHT antibody was comparable to that obtained using LIGHT-deficient B6 donor cells (Figure 4).

These results indicate that the functional blockade of LIGHT retards clonal expansion of alloreactive T cells.

LIGHT inhibition delays differentiation of alloreactive CD8^{+} T cells towards effector cells

Since LIGHT inhibition partially reduced proliferation indexes and precursor frequencies of donor alloreactive CD4^{+} and CD8^{+} T cells, we investigated whether T cell differentiation toward effector T cells had also been hampered. For this purpose, we monitored expression levels of costimulatory (BTLA, HVEM, ICOS) and differentiation (IL-7Rα, KLRG-1) molecules on host and donor CD4^{+} and CD8^{+} T cells

at five and ten days after the semiallogeneic adoptive transfer of unfractionated parental B6 splenocytes into non-irradiated F1 recipient mice.

During a viral infection, CD8 T cell differentiation toward short-lived effector T cells and memory precursor cells can be tracked with IL-7Rα (CD127) and KLRG-1 surface markers. [33] IL-7Rα is the receptor for the homeostatic cytokine IL-7 whereas KLRG-1 is a membrane glycoprotein with a C-type lectin domain and one immunoreceptor tyrosine-based inhibitory motif (ITIM) expressed in NK subsets, effector and memory T cells. [34,33] KLRG-1 and CD127 (IL-7Rα) distinguish different stages of CD8 T cell differentiation at the peak of the response, just before the contraction phase, in which 95% of responding CD8 T cells are short-lived terminal effector cells (IL-7Rα^{low}, KLRG-1^{hi}).[33] KLRG-1 is however downregulated again during the contraction phase. [35] The expression of IL-7Rα was down-regulated on donor alloreactive CD4^{+} T cells in all experimental groups at day 5 after the adoptive transfer. This was also true for donor CD8^{+} T cells in the presence of LIGHT, but not for CD8^{+} T cells in conditions of genetic or pharmacologic impairment of LIGHT: in those cases, a fair percentage of IL-7Rα-positive CD8^{+} T cells was still observed at day 5, that were however gone at day 10 post transfer (Figure 5A), suggesting that differentiation towards effector CD8 T cells was delayed in the absence of LIGHT.

KLRG-1 expression was negative in all groups at day 5 post-adoptive transfer. At day 10, a KLRG-1-positive population of alloreactive CD8^{+} T cells emerged that was more prominent in experimental groups where LIGHT contribution was genetically or pharmacologically compromised (WT to F1, isotype control, 13%; WT to F1 anti-LIGHT 3D11; 24.6%; LIGHT KO to F1, isotype control; 23.3%) (Figure 5A). This could be interpreted as a delayed entry of CD8^{+} T cells in the contraction phase in the absence of LIGHT.

Unlike naïve T cells, which are ICOS (inducible costimulatory molecule) negative, the expression of ICOS was upregulated at day 5 on most donor alloreactive CD8$^+$ T cells in isotype-treated F1 mice. However, in the absence of LIGHT, there was still a fraction of ICOS$^-$ donor CD8 T cells at day 5 (14.4% in isotype-treated controls versus 32.3% and 33.7% in anti-LIGHT-treated and LIGHT-deficient donor cells, respectively), but not anymore at day 10 (Figure 5B), again pointing at delayed maturation of effector CD8$^+$ T cells in the absence of LIGHT.

BTLA and HVEM expression were reciprocally regulated in donor CD4$^+$ and CD8$^+$ alloreactive T cells, after T cell activation and T cell expansion. Expression of BTLA is increased while that of HVEM was decreased. No significant changes in different surface markers were seen on donor alloreactive CD4$^+$ T cells when experimental groups were compared to control (Figure 5B).

Overall, these results suggest that LIGHT is required on allogeneic CD8$^+$ T cells to accelerate their differentiation.

Discussion

An increasing number of monoclonal antibodies, and to a lesser extent recombinant proteins, enter the clinical practice and provide novel therapeutic strategies for the treatment of immune related diseases. This may dramatically change in the near future the management and prognostic of these diseases. In the years to come, treatment of chronic and devastating diseases to which only palliative treatments are available will improve thanks to the substitution and complementation of chemical-based conventional therapy by biological-based approaches with therapeutic proteins.[4, 36, 37] The understanding of how TNFSF and TNFRSF molecules contribute to the development of the immune response in different models of disease is essential for translation of the research findings into clinical practice. As members of the TNF/TNFRSF family control the absolute number of effector T cells and modulate the speed of the T cell differentiation process, they dictate the frequency of memory T cells that subsequently develop into long-lived memory T cells. [4, 36]

The process of T cell activation, costimulation, clonal expansion and differentiation towards effector T cells offers potential checkpoints for immune intervention. T cell activation with no or inefficient costimulation leads to functional inactivation, unresponsiveness or impaired T cell differentiation. [38,39, 40] Therapeutics targeting the CD28/CTLA4/CD80/CD86 costimulatory pathway, which is dominated by members of the Immunoglobulin superfamily, have had a major impact on the control of allogeneic responses. CTLA4.Ig that blocks CD28 interaction with CD80 and CD86, and/or inhibition of CD40/CD40L is the most successful hits that target costimulation in the field of preclinical transplantation. [41, 42, 43,44] CTLA4.Ig (belatacept) has already been introduced in the clinic setting as a maintenance treatment that efficiently prevents rejection while reducing the metabolic side effects of conventional drugs [45]). The

treatment with CTLA4.Ig is particularly effective at low precursor frequency of alloreactive T cells that needs multiple rounds of division to reach a threshold effective to drive rejection.[46] However, CD8^{+} T cell-mediated rejection that is resistant to costimulation blockade remains an unbridgeable barrier that requires alternative strategies aiming at targeting CD8 T cell differentiation that is independent of CD4 helper cells. [47, 48]

In a previous report with a partial antagonist of the LIGHT/LTβR interaction, short-term cytotoxic response was attenuated although to a lesser extent than seen in LIGHT deficient mice. [12] Using a murine model of GvHR, in which host hematopoiesis is attacked by donor cytotoxic CD8 T cells, we now provide evidence that a fully antagonist antibody of LIGHT/HVEM and LIGHT/LTβR pathway, can down-modulate T cell responses to the same extent as that seen in LIGHT-deficient T cells. Since *in vitro* binding of LTβR to LIGHT inhibits competitively HVEM recognition of LIGHT when both receptors are expressed on the same cell, the blockade of LTβR/LIGHT by the anti-LIGHT antibody is likely to be more critical than blockade of HVEM/LIGHT. This may explain why this novel antibody (clone 3D11) fully recapitulates the phenotype of LIGHT deficient T cells while a previous reported antibody (clone 10F12) did not to achieve the same protective effect as that observed in LIGHT deficient T cells due to its partial inhibition of the LIGHT/LTβR interaction. [12]

LIGHT has been proposed by several authors as a target for immunotherapy. [49, 50,5, 26, 4, 51] Nevertheless the development of a specific anti-LIGHT reagent has been complicated until recently due to the difficulty to generate active recombinant mouse LIGHT with productive binding affinity for its receptor and functional biological activity. [52] The description, functional evaluation and validation of this anti-LIGHT antibody opens up many possibilities to study the role of LIGHT and define its

therapeutic potential in preclinical murine models of immune-related diseases (transplantation, tumor immunity and autoimmune diseases) [18, 26, 53, 54, 17, 55], as well as in other pathologies in which LIGHT has been implicated: pulmonary fibrosis subsequent to chronic lung inflammation and idiopathic pulmonary fibrosis diseases [56, 57], skin fibrosis [58]) and bone destruction through osteoclastogenesis.[59] This tool will offer advantages over the classical approaches of using LTβR.Ig recombinant fusion protein, because LTβR.Ig blocks LTαβ/LTβR in addition to LIGHT/LTβR/HVEM.[51] Administration of anti-LIGHT antibody in the above-mentioned disease models will help to clarify the contribution of LIGHT/LTβR/HVEM to the overall disease protection observed when using LTβR.Ig fusion protein. Conclusions drawn from results obtained with LTβR.Ig fusion proteins may need to be revisited because most studies utilized LTβR.Ig with a non-mutated Fc fragment of human IgG$_1$ that binds with relatively high affinity to mouse FcγRIV, the main receptor implicated in ADCC (antibody-dependent cellular cytotoxicity), [60, 61] conferring this molecule with the potential to deplete cells expressing LTβR ligands. Based on the avidity binding data of 3D11 antibody to distinct FcγR and particularly to FcγRI and FcγRIV and to a lesser extent to FcγRIII, this antibody apart from neutralizing LIGHT interactions with its receptors, it may also contribute to some extent to antibody-dependent cellular cytotoxicity.

In this work, we took advantage of a mouse model of alloreactivity in which adoptive transfer of parental B6 splenocytes into F1 recipients (BALB/c × B6) induce an allogeneic response mediated by donor CD8 cytotoxic cells that eliminate host hematopoiesis (graft versus host reaction). Donor CD4 T cells directly recognize host MHC class II alloantigens on host APC and provide cognate help to host B cells that experience transient expansion and autoantibody production. These activated B cells, as

well as the host hematopoietic compartment, are soon attacked by activated B6 donor CD8 T cells that recognize host allogeneic MHC class I alloantigens and differentiate toward cytotoxic T cells with the help provided by donor B6 CD4 T cells. [62, 32] In this mouse model, a reduced index of proliferation and precursor frequency of CD4 and CD8 T cells was observed after either pharmacological blockade or genetic ablation of LIGHT that correlated with impaired T cell expansion and differentiation towards effector T cells measured in an *in vivo* short-term assay of T cell proliferation tracked with CFSE. These observations were associated with a reduced cytotoxic activity against host F1 cells in a long-term *in vivo* assay evaluated at the peak phase of the acute graft versus host reaction, 19 days after the adoptive transfer. This indicated that the cytotoxic activity of alloreactive T cells was reduced in the absence of LIGHT. These results are in line with the first phenotypic description of LIGHT KO T cells that denoted an alteration in allogeneic T cell proliferation in these mutant mice due to a defect on IL-2 secretion by CD4 T cells. This impaired production of IL-2 would perturb clonal expansion and the help provided to CD8 T cells to promote T cell division and differentiation towards effector T cells. [19, 12]

In the context of dendritic cell activation and maturation for proper costimulation, the majority of members of the TNFSF ligands (CD40L, LTαβ, LIGHT, TL1a, CD40L, OX-40L, and 4-1BBL), are molecules transiently expressed or highly upregulated upon T cell activation that would interact with the corresponding TNFR partner molecule on APC. These activated APCs would costimulate T cells, promote their division, survival and differentiation. [2] CD40L, along with LTαβ and LIGHT, are molecules transiently expressed in the early phase of T cell activation that differentially contribute to the licensing of DC through interaction with CD40 and LTβR respectively. LTαβ/LTβR and CD40/CD40L interactions appear to be the most relevant, while LIGHT provides a

second layer of regulation. [20,63,64,65,21,22] Between LIGHT/LTβR and LIGHT/HVEM, the

predominant interaction is the former, because LTβR outcompetes HVEM due its

higher affinity for LIGHT [12]. Indirectly, LTβR signaling in stromal cells is also

important for maintaining the integrity of lymphoid tissue, which is indispensable for

the development of the immune response. [51,19, 64] Indeed, LIGHT/LTβR interaction

modulates lymph node hypertrophy by activating stromal cells expressing LTβR to

release chemokines, upregulate adhesion molecules and thus attract tissue-derived

dendritic cells to the draining lymph nodes. [28]

IL-7Rα and KLRG-1 are reciprocally modulated on CD8 T cells as they

differentiate towards effector CD8 T cells in viral infection models. Combination of

these two markers permits to follow the process of T cell differentiation. [66,33] The

absence of LIGHT (LIGHT-deficient T cells or antibody blockade of LIGHT) leads to

delayed downregulation of IL-7Rα and delayed upregulation of ICOS cells at day 5

after the adoptive transfer, suggesting that T cell differentiation was hampered in donor

alloreactive CD8 T cells in the late phase of T cell activation and clonal expansion.

Moreover, T cell differentiation toward effector cells is associated with accumulation of

KLRG-1[high] short-lived effector cells (SLECs) in the setting of transplantation and the

initiation of the contraction phase runs parallel with downregulation of KLRG-1. [35] The

increased percentage of KLRG-1^{high} SLECs in the absence of LIGHT compared to non-

treated WT donor T cells was also in line with a defect in T cell differentiation, because

CD8 T cells in the absence of LIGHT entered the contraction phase with a certain delay.

In summary, the data presented here provide evidence for the therapeutic use of

LIGHT inhibitors to dampen immune responses via attenuation of donor allogeneic

CD8 T cell responses.

Material and Methods

Animals

Eight to twelve weeks-old female C57BL/6 (H-2^b, B6, CD45.2), BALB/c (H-2^d), CB6F1 (BALB/c × C57BL/6 F1) mice (H-2$^{d/b}$) and LIGHT-deficient mice (LIGHT$^{-/-}$) backcrossed more than fifteen generations onto C57BL/6 background were bred at the animal facility of the University of Leon (Spain).[19] All experiments with rodents were performed in accordance and following animal protocols specifically approved for the Ethical Committee for Animal Research of the School of Veterinary Medicine (University of Leon), the Animal Welfare Committee of University of Alcala de Henares (Madrid) and followed the European Guidelines for Animal Care and Use of Laboratory Animals.

Monoclonal antibodies and surface plasmon resonance

LIGHT-deficient mice were immunized intraperitoneally with 0.25 ml of a 1:1.2 mixture of 5-10 × 10^6 of GFP-tagged mouse LIGHT transduced into NIH-3T3 cells in Freund's Incomplete Adjuvant (Sigma).[12] Six weeks after the priming immunization, mice received an intravenous booster injection of LIGHT-transduced cells in saline. The immortalization of mouse B cells with myeloma cell line X63 Ag8.653 was previously described[67] and supernatants of the heterohybridomas secreting rat monoclonal antibodies were tested ten days after for their specificity against LIGHT transduced cells by flow cytometry.

Flag-Foldon-tagged soluble mouse LIGHT (FF-LIGHT)[68], soluble FF-control protein (FF-Ctrl) and LTβR.human IgG1.Fc (LTβR.Ig) were produced and purified as previously reported.[69] Recombinant mouse HVEM.mouse IgG2a.Fc (HVEM.Ig)

produced in insect cells was a gift from Genentech. Commercially available mouse

IgG$_{2a}$ and human IgG$_1$ were used as matched isotype control for the recombinant

proteins.

For the screening of anti-LIGHT antibodies displaying antagonist activity and

therefore capable to prevent LIGHT interaction with its receptors, 2.5×10^5 LIGHT-

transduced NIH-3T3 cells were incubated with a saturating amount of isotype matched

control or anti-mouse LIGHT mAbs for 30 min at room temperature (2 μg/ml). In the

presence of competitor antibody, cells were then further incubated for 2 h at 37ºC with

an optimal dilution of either HVEM-Ig (5 μg/well) or LTβR-Ig (1 μg/well) in a final

volume of 100 μl. After a washing step, the reaction was developed with the appropriate

biotinylated conjugates (biotinylated rat anti-mouse IgG$_{2a}$ isotype specific mAb, clone

R19-15, BD Biosciences or mouse anti-human IgG Fc fragment, Jackson

ImmunoResearch), followed by allophycocyanin-coupled to streptavidin.

The BIACORE 3000 system, sensor chip CM5, surfactant P20, amine coupling

kit containing N-hydroxysuccinimide (NHS) and N-Ethyl-N'-dimethylaminopropyl

carbodiimide (EDC), were from BIACORE (Upsala, Sweden). All biosensor assays

were performed with HEPES-buffered saline (HBS-EP) as running buffer (10 mM

HEPES, 150 mM sodium acetate, 3 mM magnesium acetate, 3.4 mM EDTA and

0.005% surfactant P20, pH 7.4). The different compounds were dissolved into running

buffer. [70]

FcγRI (CD64), FcγRIIB (CD32), FcγRIIIA (CD16) and FcγRIV (CD16.2) were

immobilized at 50 μg/ml in formate buffer, pH 4.3 by injection onto the EDC/NHS-

activated surface of a CM5-type sensor chip until a signal of approximately 6000 RU

was obtained. Free activated sites of the matrix were saturated by injection of 20 μL

of ethanolamine hydrochloride pH 8.5. All the binding experiments were carried out at

25°C with a constant flow rate of 30 μl/min. Different concentrations of mouse anti-LIGHT (clone 3D11) were injected for 3 min followed by a dissociation phase of 3 min. The sensor chip surface was regenerated after each experiment by injection of 20 μl of 10 mM NaOH.

The kinetic parameters were calculated using the BIAeval 4.1 software on a personal computer. Global analysis was performed using the simple Langmuir binding model. The specific binding profiles were obtained after subtracting the response signal from the channel control (ethanolamine) and from blank buffer injection. The fitting to each model was judged by the reduced chi square and randomness of residue distribution.

Flow cytometry

Mouse anti-mouse LIGHT (3D11, mouse IgG$_{2b}$) and mouse IgG$_{2b}$ isotype control (MPC-11, Biolegend) mAbs were labeled with Hilyte-Fluor 647 according to the manufacturer's protocol (Anaspec Inc). CD4 (GK1.5), CD8α (53-6.7), Ly6G (1A8), CD19 (6D5), ICOS (7E.17G9), CD127 (IL-7Rα, A7R34), and killer cell lectin-like receptor subfamily G, member 1 (KLRG-1, MAFA) mAbs were all purchased from Biolegend (USA). To distinguish hematopoietic cells of donor parental B6 and host F1, cells from distinct hematopoietic compartments were stained with FITC-conjugated anti–H-2^d (SF1-1.1) and Alexa 647-conjugated anti-H-2^b (AF6-88.5). Rat monoclonal antibodies anti-mouse BTLA (CD272, clone 4G12b) [71] and anti-mouse HVEM (CD250, clone 10F3) [29] were produced and labeled in house and used in this study.

Fc gamma receptors were blocked by incubating cell suspensions with 2 μg/ml of blocking anti-FcγR (rat IgG$_{2b}$ anti-FcγRII/III mAb, clone 2.4G2) before staining with fluorochrome-labeled antibodies. [72] Dead cells were excluded from the acquisition gate

by staining with propidium iodide. Samples were acquired on a Cyan 9 cytometer (Beckman Coulter, Miami, FL, USA) and data analysis was performed using WinList version 7.0 (Verity Software House, Topsham, ME, USA).

In vitro polyclonal T cell activation to induce LIGHT expression

Naïve C57BL/6 splenocytes were polyclonally activated *in vitro* with Phorbol Myristate Acetate (PMA, 100 ng/ml) plus ionomycin (500 ng/ml) or were left untreated for 5 h at 37 ºC. The transient expression of LIGHT was analyzed on resting and polyclonally activated T cells (2×10^5 cells / well) stained with 1 µg/well of Hylite 647-labeled anti-LIGHT mAb (clone 3D11) or Hylite 647-labeled isotype-matched mouse IgG_{2b} (MPC-11) during the time of incubation, using a lineage cocktail to gate out all cells with phagocytic phenotype ($CD19^+$ $Ly6G^+$ $CD11c^+$) that take up the antibody unspecifically.

In vivo proliferative assay of CFSE-labeled donor alloreactive T cells

70×10^6 of B6 WT or LIGHT-deficient unfractionated splenocytes were labeled with 5 µM carboxyfluorescein diacetate succinmidyl ester (CFSE) and adoptively transferred into non-irradiated F1 recipients, according to Lyons et al. [73] The day of the adoptive transfer, recipient mice were treated with 1 mg (~40 mg/kg) of isotype-control (mouse IgG_{2b}) or mouse anti-mouse LIGHT (3D11) mAb. Three days later, the Proliferative Index (PI) and Precursor Frequency (PF) of alloreactive $CD4^+$ and $CD8^+$ T cells were determined by deconvoluting and analyzing the reduced CFSE fluorescence using the ModFit LT 4.1 version (Verity software, ME). PI, is a measure of the increase in cell number over the course of the assay. PF returns the fraction of cells in the initial population that responded to the stimulus by proliferating. The location of each generation of cells is represented by a unique peak color.

***In vivo* murine model of Graft versus Host Reaction**

Naïve C57BL/6 donor splenocytes (70×10^6 cells) were intravenously transferred into recipient F1 mice that were treated intraperitoneally at day 0 with 1 mg/mouse of anti-LIGHT (3D11) or isotype mouse IgG$_{2b}$ control antibody. In a third experimental group, F1 recipient mice were inoculated with 70×10^6 splenocytes from LIGHT-deficient mice in B6 background and treated with 1 mg isotype control. Finally, a syngeneic control group was included in which 70×10^6 syngeneic F1 splenocytes were injected into non-irradiated F1 recipients. The absolute number of hematopoietic cells in primary and secondary lymphoid organs was recorded nineteen days after the adoptive transfer.

Statistical analysis

Collected data from experimental and control groups were analyzed using Graph Pad prism Version 5. Statistical significance was assessed using the non-parametric Mann-Whitney *t* test or Student´s t test with Welch´s correction to compare the means among groups. A *p* value less than 0.05 was considered statistically significant.

Figure legends

Figure 1: Effective blockade of LIGHT/LTβR and LIGHT/HVEM interactions using a mouse anti-mouse LIGHT monoclonal antibody

2.5×10^5 NIH-3T3 cells transduced with GFP-tagged murine LIGHT (blue solid lines) or GFP-transduced NIH-3T3 cells (red solid lines) were incubated with a mouse anti-mouse LIGHT mAb 3D11. After an incubation step, antibody binding was revealed with biotinylated rat anti-mouse IgG$_{2b}$ followed by allophycocyanin-coupled streptavidin **(A, upper panel)**. To further demonstrate the specificity of the anti-LIGHT mAb, 1 μg/well of Hilyte 647-labeled anti-LIGHT mAb alone (blue solid line) or 1 μg/well Hilyte 647-labeled anti-LIGHT mAb preincubated with 2 μg/well FF-LIGHT fusion protein (red solid line) were added to LIGHT-GFP transduced NIH 3T3 cells **(A, lower panel)**.

(B) 2.5×10^5 LIGHT-transduced NIH-3T3 cells were pre-incubated for 30 min at room temperature with anti-LIGHT mAb (clone 3D11, blue solid lines) or mouse IgG$_{2b}$ isotype control (red dotted lines), then stained in the presence of the antibodies with LTβR-Ig (upper panel) or HVEM-Ig (lower panel). Binding of LTβR-Ig was revealed with biotinylated anti-hIgG and binding of HVEM-Ig was revealed with biotinylated anti-mouse IgG$_{2a}$, both followed by allophycocyanin-coupled streptavidin. The baseline background staining is represented by hIgG$_1$ Fc fragment (upper panel, black solid lines) to LIGHT-transduced NIH-3T3 cells or the binding of the Fc fragment mIgG$_{2a}$ (lower panel, black solid lines). The mean fluorescence intensity (MFI) is indicated in each plot.

(C) Serial dilutions of anti-LIGHT mAb (clone 3D11) were preincubated with a fixed amount of 1 μg/well of soluble recombinant FF control (red solid line) or FF-LIGHT

protein (black solid line). Then, 1×10^5 LIGHT-transduced NIH-3T3 were added to the reaction. After a washing step, a biotinylated rat anti-mouse IgG$_{2b}$ conjugate was further incubated and developed by SA-APC. The mean fluorescence intensity (MFI) binding of anti-LIGHT mAb to LIGHT transduced cells in the presence of FF control or FF-LIGHT is plotted.

(D) The association and dissociation constant rates of 3D11 mAb binding to distinct immobilized mouse FcγR were calculated by surface plasmon resonance and from those values the equilibrium dissociation constant K$_D$ for each of them was determined.

Figure 2: Mouse LIGHT is rapidly upregulated on activated T cells after polyclonal stimulation

(A) Naïve C57BL/6 splenocytes (2×10^5) were left untreated or were stimulated with PMA (100 ng/ml) and ionomycin (500 ng/ml) for 5 h. 1 μg/well Hilyte-647-labeled anti mouse LIGHT (3D11) or Hylite-647-labeled isotype control mouse IgG$_{2b}$ were added to cultures during incubation in a final volume of 250 μl. A lineage cocktail to gate out CD19$^+$ CD11c$^+$ Ly6G$^+$ cells was included in the staining. The expression of mouse LIGHT was then analyzed on live resting and activated CD4 and CD8 T cells.

(B) The expression of mouse LIGHT on the surface of resting or PMA plus ionomycin activated CD4$^+$ and CD8$^+$ T cells was also determined with the anti-LIGHT antibody pre-incubated with Flag-Foldon LIGHT (FF-LIGHT) (2 μg FF-LIGHT per well). The percentage of each population is represented in each quadrant. PMA: Phorbol Myristate Acetate. Iono: Ionomycin.

Figure 3: LIGHT blockade attenuated the course of graft versus host reaction in a mouse model of alloreactivity

Semiallogeneic splenocytes from C57BL/6 (70×10^6) were intravenously transferred into non irradiated CB6F1 recipients, which were treated with 1 mg of isotype mouse IgG$_{2b}$ control (red squares) or anti-LIGHT mAb (3D11, green triangles) at day 0. In a third experimental group, semiallogeneic splenocytes from LIGHT-deficient mice (70×10^6) were injected into CB6F1 mice (orange diamonds). The fourth group represents the syngeneic control group, in which 70×10^6 F1 splenocytes was injected into F1 recipients (blue circles). The absolute number of host bone marrow (**A**), thymocytes (**B**), double positive thymocytes (**C**) and splenocytes (**D**) was determined nineteen days after the adoptive transfer. Data represent a pool of three independent experiments. Statistical significance is indicated as follows: *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, and ns, non-significant.

Figure 4: The proliferation index and frequency of donor CD4$^+$ and CD8$^+$ alloreactive T cells is altered after LIGHT blockade or in LIGHT-deficient T cells.

70×10^6 of CFSE (carboxyfluorescein diacetate succinmidyl ester)-labeled B6 WT or CFSE-labeled B6 LIGHT-deficient splenocytes were adoptively transferred into non-irradiated F1 recipients and treated with 1 mg of isotype-control (mouse IgG$_{2b}$) or mouse anti-mouse LIGHT (3D11) mAb at day 0. Three days later, the Proliferative Index (PI) and percentage of Precursor Frequency (PF) of donor alloreactive CD4$^+$ and CD8$^+$ T cells were determined using the ModFit LT software. Black line profile deconvoluted into cells that had divided once (green), twice (light violet), 3x (light blue), 4x (yellow), 5x (red), 6x (dark violet) and 7x or more (light green). X-axis

represents CFSE fluorescence on a log scale and Y axis indicates cell counts (number of events).

Figure 5: Altered expression of differentiation (IL-7Rα and KLRG-1) and costimulatory (ICOS) surface markers is associated with delayed donor CD8 T cell differentiation in the absence of LIGHT

The pattern of expression of costimulatory and differentiation markers was assessed at days 5 and 10 after adoptive transfer of 70×10^6 of donor syngeneic or allogeneic B6 WT or B6 LIGHT-deficient splenocytes into F1 recipients. Recipients were treated on the day of the adoptive transfer with 1 mg of isotype control mouse IgG$_{2b}$ or anti-LIGHT mAb, clone 3D11. The expression of differentiation markers **(A)** and costimulatory molecules **(B)** was assessed by flow cytometry on host (blue lines) and donor (red solid lines) CD4$^+$ and CD8$^+$ T cells
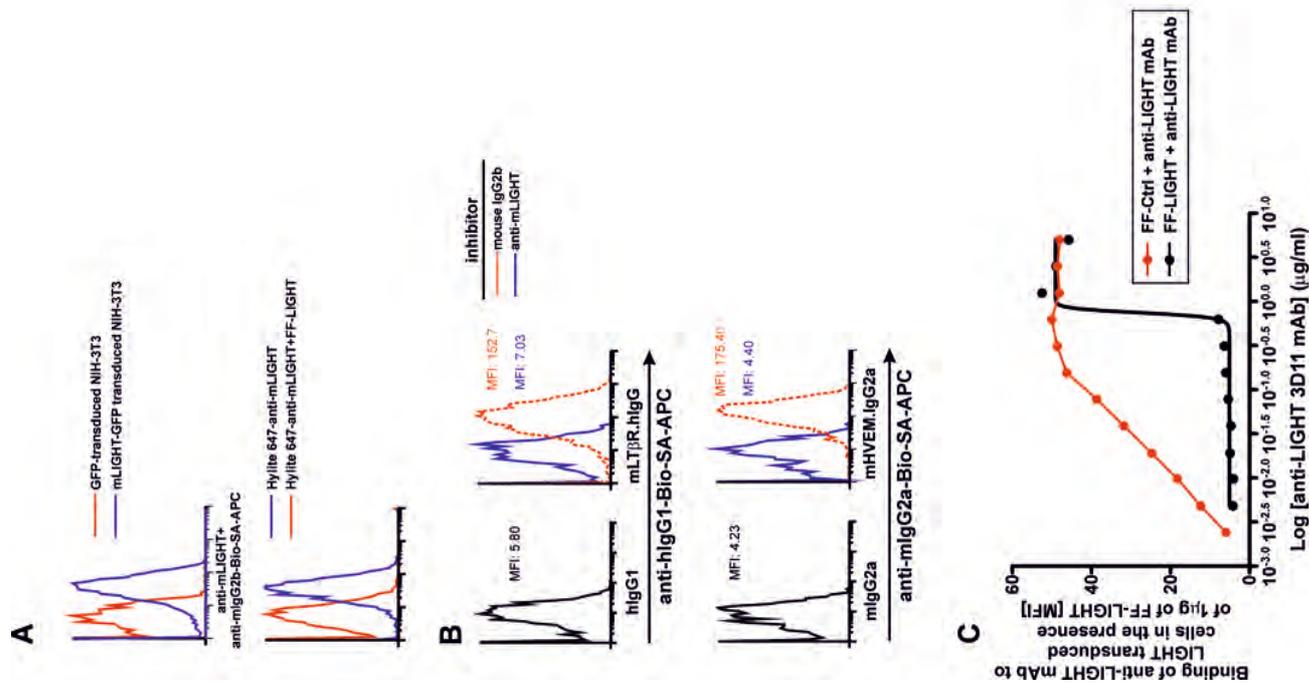
Bibliographic references

1.   Croft M. The role of TNF superfamily members in T-cell function and diseases. NatRevImmunol 2009; 9:271-85.
2.   Croft M. The TNF family in T cell differentiation and function--unanswered questions and future directions. Semin Immunol 2014; 26:183-90.
3.   Sedy J, Bekiaris V, Ware CF. Tumor Necrosis Factor Superfamily in Innate Immunity and Inflammation. Cold Spring Harb Perspect Biol 2014; 7.
4.   Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. Nat Rev Drug Discov 2013; 12:147-68.
5.   Ware CF. Targeting lymphocyte activation through the lymphotoxin and LIGHT pathways. ImmunolRev 2008; 223:186-201.
6.   Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, Ruben S, Murphy M, Eisenberg RJ, Cohen GH, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity 1998; 8:21-30.
7.   Rooney IA, Butrovich KD, Glass AA, Borboroglu S, Benedict CA, Whitbeck JC, Cohen GH, Eisenberg RJ, Ware CF. The lymphotoxin-beta receptor is necessary and sufficient for LIGHT-mediated apoptosis of tumor cells. JBiolChem 2000; 275:14307-15.
8.   Zhai Y, Guo R, Hsu TL, Yu GL, Ni J, Kwon BS, Jiang GW, Lu J, Tan J, Ugustus M, et al. LIGHT, a novel ligand for lymphotoxin beta receptor and TR2/HVEM induces apoptosis and suppresses in vivo tumor formation via gene transfer. JClinInvest 1998; 102:1142-51.

9.   Granger SW, Butrovich KD, Houshmand P, Edwards WR, Ware CF. Genomic characterization of LIGHT reveals linkage to an immune response locus on chromosome 19p13.3 and distinct isoforms generated by alternate splicing or proteolysis. JImmunol 2001; 167:5122-8.

10.   Zhang J, Salcedo TW, Wan X, Ullrich S, Hu B, Gregorio T, Feng P, Qi S, Chen H, Cho YH, et al. Modulation of T-cell responses to alloantigens by TR6/DcR3. JClinInvest 2001; 107:1459-68.

11.   Yu KY, Kwon B, Ni J, Zhai Y, Ebner R, Kwon BS. A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. JBiolChem 1999; 274:13733-6.

12.   del Rio ML, Fernandez-Renedo C, Scheu S, Pfeffer K, Shintani Y, Kronenberg M, Chaloin O, Schneider P, Rodriguez-Barbosa JI. Therapeutic blockade of LIGHT interaction with herpesvirus entry mediator and lymphotoxin beta receptor attenuates in vivo cytotoxic allogeneic responses. Transplantation 2014; 98:1165-74.

13.   Morel Y, Schiano de Colella JM, Harrop J, Deen KC, Holmes SD, Wattam TA, Khandekar SS, Truneh A, Sweet RW, Gastaut JA, et al. Reciprocal expression of the TNF family receptor herpes virus entry mediator and its ligand LIGHT on activated T cells: LIGHT down-regulates its own receptor. JImmunol 2000; 165:4397-404.

14.   Force WR, Walter BN, Hession C, Tizard R, Kozak CA, Browning JL, Ware CF. Mouse lymphotoxin-beta receptor. Molecular genetics, ligand binding, and expression. JImmunol 1995; 155:5280-8.

15.   Croft M, Duan W, Choi H, Eun SY, Madireddi S, Mehta A. TNF superfamily in inflammatory disease: translating basic insights. Trends Immunol 2012; 33:144-52.

16.   Fan K, Wang H, Wei H, Zhou Q, Kou G, Hou S, Qian W, Dai J, Li B, Zhang Y, et al. Blockade of LIGHT/HVEM and B7/CD28 signaling facilitates long-term islet graft survival with development of allospecific tolerance. Transplantation 2007; 84:746-54.

17.   Ye Q, Fraser CC, Gao W, Wang L, Busfield SJ, Wang C, Qiu Y, Coyle AJ, Gutierrez-Ramos JC, Hancock WW. Modulation of LIGHT-HVEM costimulation prolongs cardiac allograft survival. JExpMed 2002; 195:795-800.

18.   Tamada K, Shimozaki K, Chapoval AI, Zhu G, Sica G, Flies D, Boone T, Hsu H, Fu YX, Nagata S, et al. Modulation of T-cell-mediated immunity in tumor and graft-versus-host disease models through the LIGHT co-stimulatory pathway. NatMed 2000; 6:283-9.

19.   Scheu S, Alferink J, Potzel T, Barchet W, Kalinke U, Pfeffer K. Targeted disruption of LIGHT causes defects in costimulatory T cell activation and reveals cooperation with lymphotoxin beta in mesenteric lymph node genesis. JExpMed 2002; 195:1613-24.

20.   Tamada K, Shimozaki K, Chapoval AI, Zhai Y, Su J, Chen SF, Hsieh SL, Nagata S, Ni J, Chen L. LIGHT, a TNF-like molecule, costimulates T cell proliferation and is required for dendritic cell-mediated allogeneic T cell response. JImmunol 2000; 164:4105-10.

21.   Morel Y, Truneh A, Sweet RW, Olive D, Costello RT. The TNF superfamily members LIGHT and CD154 (CD40 ligand) costimulate induction of dendritic cell maturation and elicit specific CTL activity. JImmunol 2001; 167:2479-86.

22.   Holmes TD, Wilson EB, Black EV, Benest AV, Vaz C, Tan B, Tanavde VM, Cook GP. Licensed human natural killer cells aid dendritic cell maturation via TNFSF14/LIGHT. Proceedings of the National Academy of Sciences of the United States of America 2014; 111:E5688-96.

23.   Wan X, Zhang J, Luo H, Shi G, Kapnik E, Kim S, Kanakaraj P, Wu J. A TNF family member LIGHT transduces costimulatory signals into human T cells. JImmunol 2002; 169:6813-21.

24.   Shi G, Luo H, Wan X, Salcedo TW, Zhang J, Wu J. Mouse T cells receive costimulatory signals from LIGHT, a TNF family member. Blood 2002; 100:3279-86.

25.   Granger SW, Rickert S. LIGHT-HVEM signaling and the regulation of T cell-mediated immunity. Cytokine Growth Factor Rev 2003; 14:289-96.
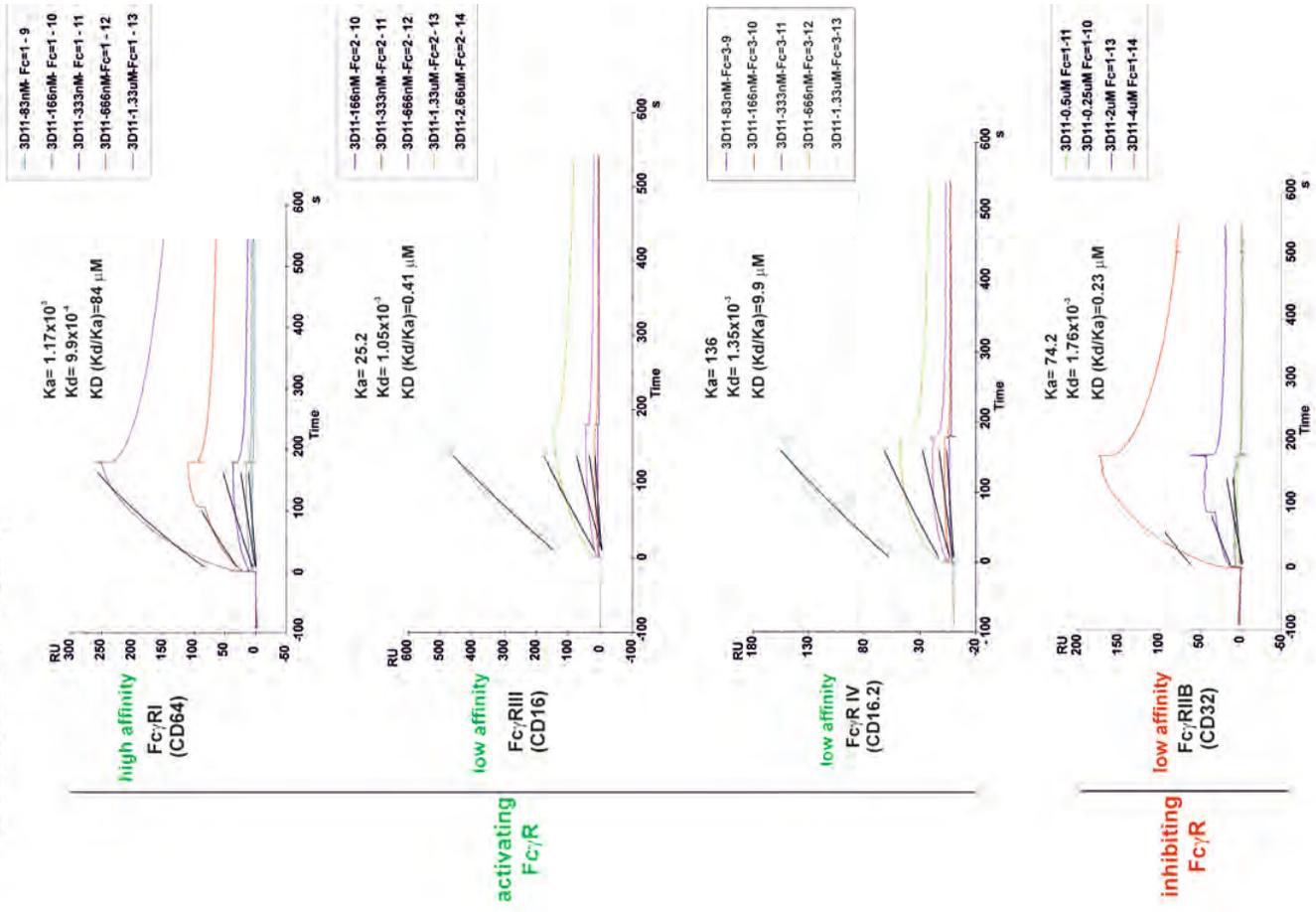
26.   Xu Y, Flies AS, Flies DB, Zhu G, Anand S, Flies SJ, Xu H, Anders RA, Hancock WW, Chen L, et al. Selective targeting of the LIGHT-HVEM costimulatory system for the treatment of graft-versus-host disease. Blood 2007; 109:4097-104.

27.   Chattopadhyay PK, Yu J, Roederer M. A live-cell assay to detect antigen-specific CD4+ T cells with diverse cytokine profiles. NatMed 2005; 11:1113-7.

28.   Zhu M, Yang Y, Wang Y, Wang Z, Fu YX. LIGHT regulates inflamed draining lymph node hypertrophy. JImmunol 2011; 186:7156-63.

29.   Del Rio ML, Jones ND, Buhler L, Norris P, Shintani Y, Ware CF, Rodriguez-Barbosa JI. Selective blockade of herpesvirus entry mediator-B and T lymphocyte attenuator pathway ameliorates acute graft-versus-host reaction. JImmunol 2012; 188:4885-96.

30.   Fan Z, Yu P, Wang Y, Wang Y, Fu ML, Liu W, Sun Y, Fu YX. NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. Blood 2006; 107:1342-51.

31.   Pulaiev RA, Puliaeva IA, Ryan AE, Via CS. The Parent-into-F1 Model of Graft-vs-Host Disease as a Model of In Vivo T Cell Function and Immunomodulation. CurrMedChemImmunolEndocrMetab Agents 2005; 5:575-83.

32.   Tschetter JR, Mozes E, Shearer GM. Progression from acute to chronic disease in a murine parent-into-F1 model of graft-versus-host disease. JImmunol 2000; 165:5987-94.

33.   Sarkar S, Kalia V, Haining WN, Konieczny BT, Subramaniam S, Ahmed R. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. The Journal of experimental medicine 2008; 205:625-40.

34.   Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. NatImmunol 2003; 4:1191-8.

35.   Ferrer IR, Wagener ME, Song M, Ford ML. CD154 blockade alters innate immune cell recruitment and programs alloreactive CD8+ T cells into KLRG-1(high) short-lived effector T cells. PloS one 2012; 7:e40559.

36.   Ware CF. Protein therapeutics targeted at the TNF superfamily. Advances in pharmacology (San Diego, Calif) 2013; 66:51-80.

37.   Page EK, Dar WA, Knechtle SJ. Biologics in organ transplantation. TransplInt 2012; 25:707-19.

38.   Miller JF, Morahan G. Peripheral T cell tolerance. AnnuRevImmunol 1992; 10:51-69.

39.   van Parijs L, Perez VL, Abbas AK. Mechanisms of peripheral T cell tolerance. NovartisFoundSymp 1998; 215:5-14.

40.   Schwartz RH. T cell anergy. AnnuRevImmunol 2003; 21:305-34.

41.   Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998; 393:480-3.

42.   Judge TA, Wu Z, Zheng XG, Sharpe AH, Sayegh MH, Turka LA. The role of CD80, CD86, and CTLA4 in alloimmune responses and the induction of long-term allograft survival. JImmunol 1999; 162:1947-51.

43.   Hargreaves RE, Monk NJ, Jurcevic S. Selective depletion of activated T cells: the CD40L-specific antibody experience. Trends Mol Med 2004; 10:130-5.

44.   Tran HM, Nickerson PW, Restifo AC, Ivis-Woodward MA, Patel A, Allen RD, Strom TB, O'Connell PJ. Distinct mechanisms for the induction and maintenance of allograft tolerance with CTLA4-Fc treatment. Journal of immunology (Baltimore, Md : 1950) 1997; 159:2232-9.

45.   Wekerle T, Grinyo JM. Belatacept: from rational design to clinical application. TransplInt 2012; 25:139-50.

46.   Ford ML, Larsen CP. Translating costimulation blockade to the clinic: lessons learned from three pathways. Immunological reviews 2009; 229:294-306.

47.   Williams MA, Trambley J, Ha J, Adams AB, Durham MM, Rees P, Cowan SR, Pearson TC, Larsen CP. Genetic characterization of strain differences in the ability to mediate CD40/CD28-independent rejection of skin allografts. JImmunol 2000; 165:6849-57.

48.   Trambley J, Bingaman AW, Lin A, Elwood ET, Waitze SY, Ha J, Durham MM, Corbascio M, Cowan SR, Pearson TC, et al. Asialo GM1(+) CD8(+) T cells play a critical role in costimulation blockade-resistant allograft rejection. JClinInvest 1999; 104:1715-22.

49.   Doherty TA, Croft M. Therapeutic potential of targeting TNF/TNFR family members in asthma. Immunotherapy 2011; 3:919-21.

50.   Cheung TC. Modulation of T cell proliferation through the LIGHT-HVEM-BTLA cosignaling pathway. Recent Pat DNA Gene Seq 2009; 3:177-82.

51.   Gommerman JL, Browning JL. Lymphotoxin/light, lymphoid microenvironments and autoimmune disease. NatRevImmunol 2003; 3:642-55.

52.   Tsuji I, Iwamoto K, Shintani Y. Effective expression and purification of bioactive recombinant soluble LIGHT. Methods in molecular biology (Clifton, NJ) 2014; 1155:201-13.

53.   Wang J, Lo JC, Foster A, Yu P, Chen HM, Wang Y, Tamada K, Chen L, Fu YX. The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. JClinInvest 2001; 108:1771-80.

54.   Anand S, Wang P, Yoshimura K, Choi IH, Hilliard A, Chen YH, Wang CR, Schulick R, Flies AS, Flies DB, et al. Essential role of TNF family molecule LIGHT as a cytokine in the pathogenesis of hepatitis. JClinInvest 2006; 116:1045-51.

55.   Fava RA, Notidis E, Hunt J, Szanya V, Ratcliffe N, Ngam-Ek A, De Fougerolles AR, Sprague A, Browning JL. A role for the lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. Journal of immunology (Baltimore, Md : 1950) 2003; 171:115-26.

56.   Herro R, Da Silva Antunes R, Aguilera AR, Tamada K, Croft M. Tumor necrosis factor superfamily 14 (LIGHT) controls thymic stromal lymphopoietin to drive pulmonary fibrosis. The Journal of allergy and clinical immunology 2015.

57.   Doherty TA, Soroosh P, Khorram N, Fukuyama S, Rosenthal P, Cho JY, Norris PS, Choi H, Scheu S, Pfeffer K, et al. The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling. NatMed 2011; 17:596-603.

58.   Herro R, Antunes Rda S, Aguilera AR, Tamada K, Croft M. The Tumor Necrosis Factor Superfamily Molecule LIGHT Promotes Keratinocyte Activity and Skin Fibrosis. The Journal of investigative dermatology 2015; 135:2109-18.

59.   Brunetti G, Rizzi R, Oranger A, Gigante I, Mori G, Taurino G, Mongelli T, Colaianni G, Di Benedetto A, Tamma R, et al. LIGHT/TNFSF14 increases osteoclastogenesis and decreases osteoblastogenesis in multiple myeloma-bone disease. Oncotarget 2014; 5:12950-67.

60.   Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. Blood 2012; 119:5640-9.

61.   Bournazos S, DiLillo DJ, Ravetch JV. The role of Fc-FcgammaR interactions in IgG-mediated microbial neutralization. The Journal of experimental medicine 2015.

62.   Soloviova K, Puliaiev M, Foster A, Via CS. The parent-into-F1 murine model in the study of lupus-like autoimmunity and CD8 cytotoxic T lymphocyte function. Methods in molecular biology (Clifton, NJ) 2012; 900:253-70.

63.   Summers-DeLuca LE, McCarthy DD, Cosovic B, Ward LA, Lo CC, Scheu S, Pfeffer K, Gommerman JL. Expression of lymphotoxin-alphabeta on antigen-specific T cells is required for DC function. The Journal of experimental medicine 2007; 204:1071-81.

64.   Gommerman JL, Summers dL. LTbetaR and CD40: working together in dendritic cells to optimize immune responses. ImmunolRev 2011; 244:85-98.

65.   Del Rio ML, Schneider P, Fernandez-Renedo C, Perez-Simon JA, Rodriguez-Barbosa JI. LIGHT/HVEM/LTbetaR Interaction as a Target for the Modulation of the Allogeneic Immune Response in Transplantation. AmJTransplant 2013; 13:541-51.
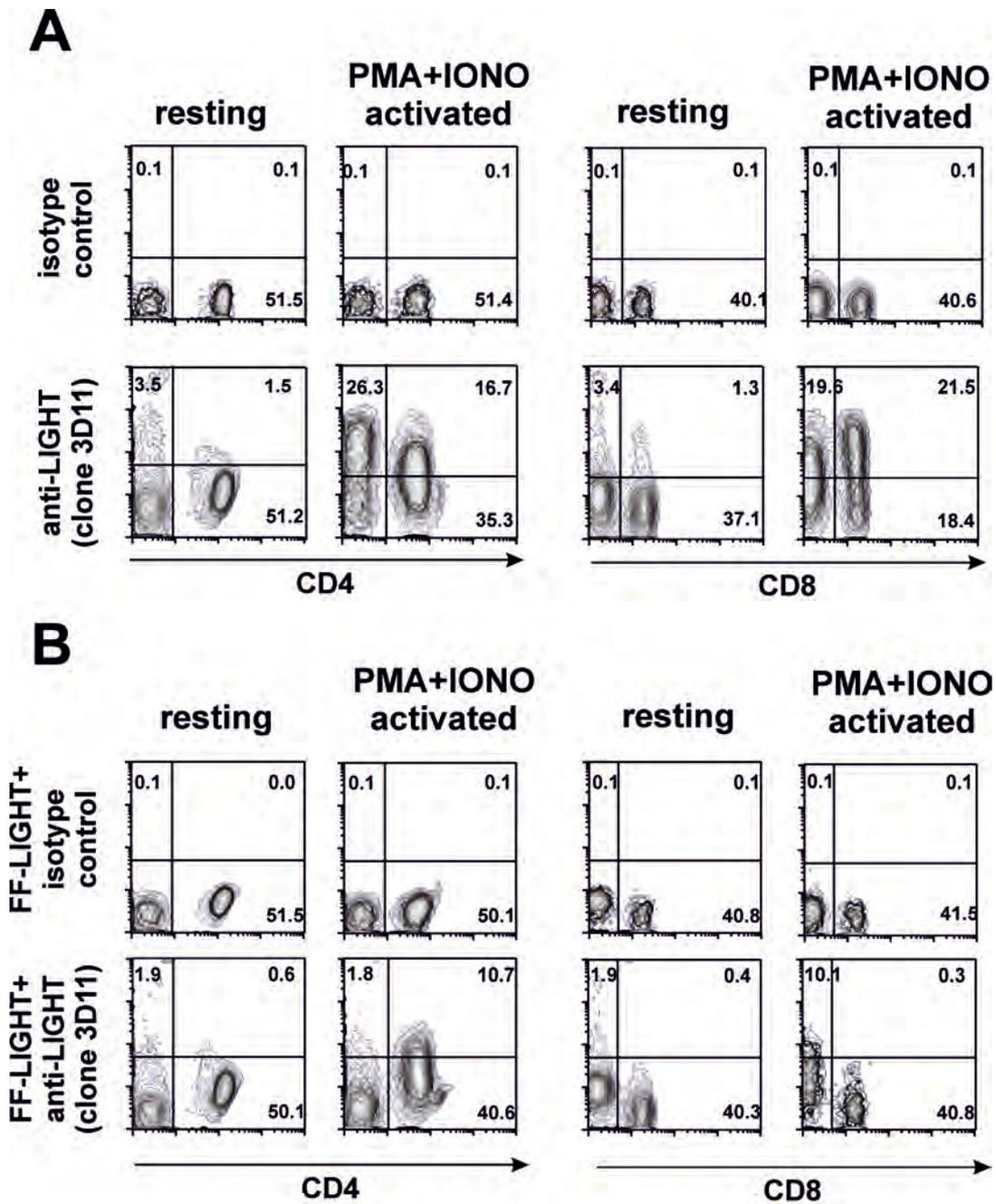
66.   Schenk AD, Gorbacheva V, Rabant M, Fairchild RL, Valujskikh A. Effector functions of donor-reactive CD8 memory T cells are dependent on ICOS induced during division in cardiac grafts. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2009; 9:64-73.

67.   Rodriguez-Barbosa JI, Gutierrez Martin CB, Tascon RI, Suarez J, Rodriguez Ferri EF. Evidence obtained with monoclonal antibodies that O antigen is the major antigen responsible for the cross-reactivities between serotypes 4 and 7 of Actinobacillus (Haemophilus) pleuropneumoniae. ClinDiagnLab Immunol 1995; 2:563-8.

68.   Ito T, Iwamoto K, Tsuji I, Tsubouchi H, Omae H, Sato T, Ohba H, Kurokawa T, Taniyama Y, Shintani Y. Trimerization of murine TNF ligand family member LIGHT increases the cytotoxic activity against the FM3A mammary carcinoma cell line. ApplMicrobiolBiotechnol 2011; 90:1691-9.

69.   Schneider P, Willen L, Smulski CR. Tools and techniques to study ligand-receptor interactions and receptor activation by TNF superfamily members. Methods in enzymology 2014; 545:103-25.

70.   Nguyen HH, Park J, Kang S, Kim M. Surface plasmon resonance: a versatile technique for biosensor applications. Sensors (Basel, Switzerland) 2015; 15:10481-510.

71.   Del Rio ML, Kaye J, Rodriguez-Barbosa JI. Detection of protein on BTLA(low) cells and in vivo antibody-mediated down-modulation of BTLA on lymphoid and myeloid cells of C57BL/6 and BALB/c BTLA allelic variants. Immunobiology 2010; 215:570-8.

72.   Unkeless JC. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. JExpMed 1979; 150:580-96.

73.   Lyons AB. Analysing cell division in vivo and in vitro using flow cytometric measurement of CFSE dye dilution. JImmunolMethods 2000; 243:147-54.
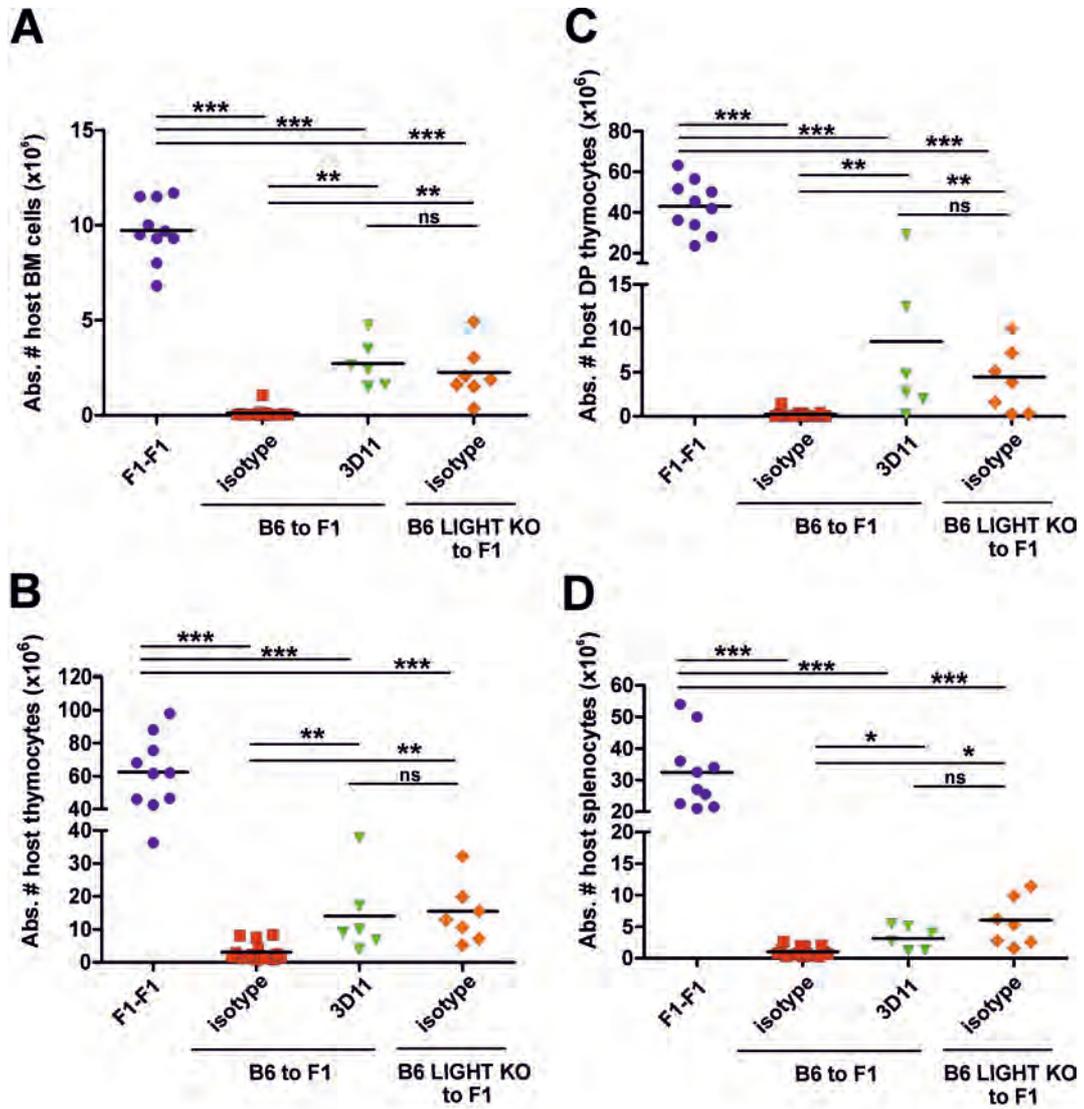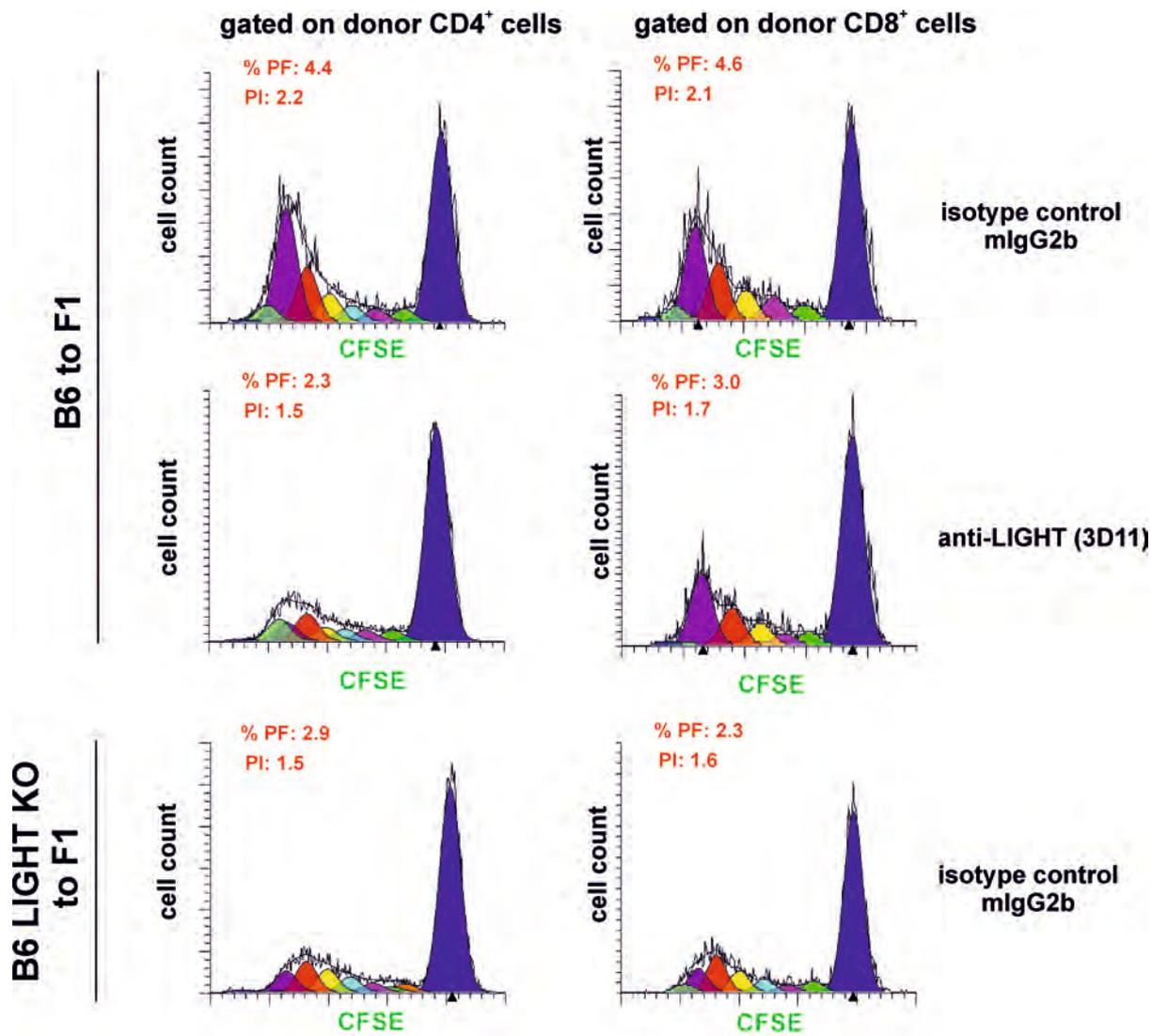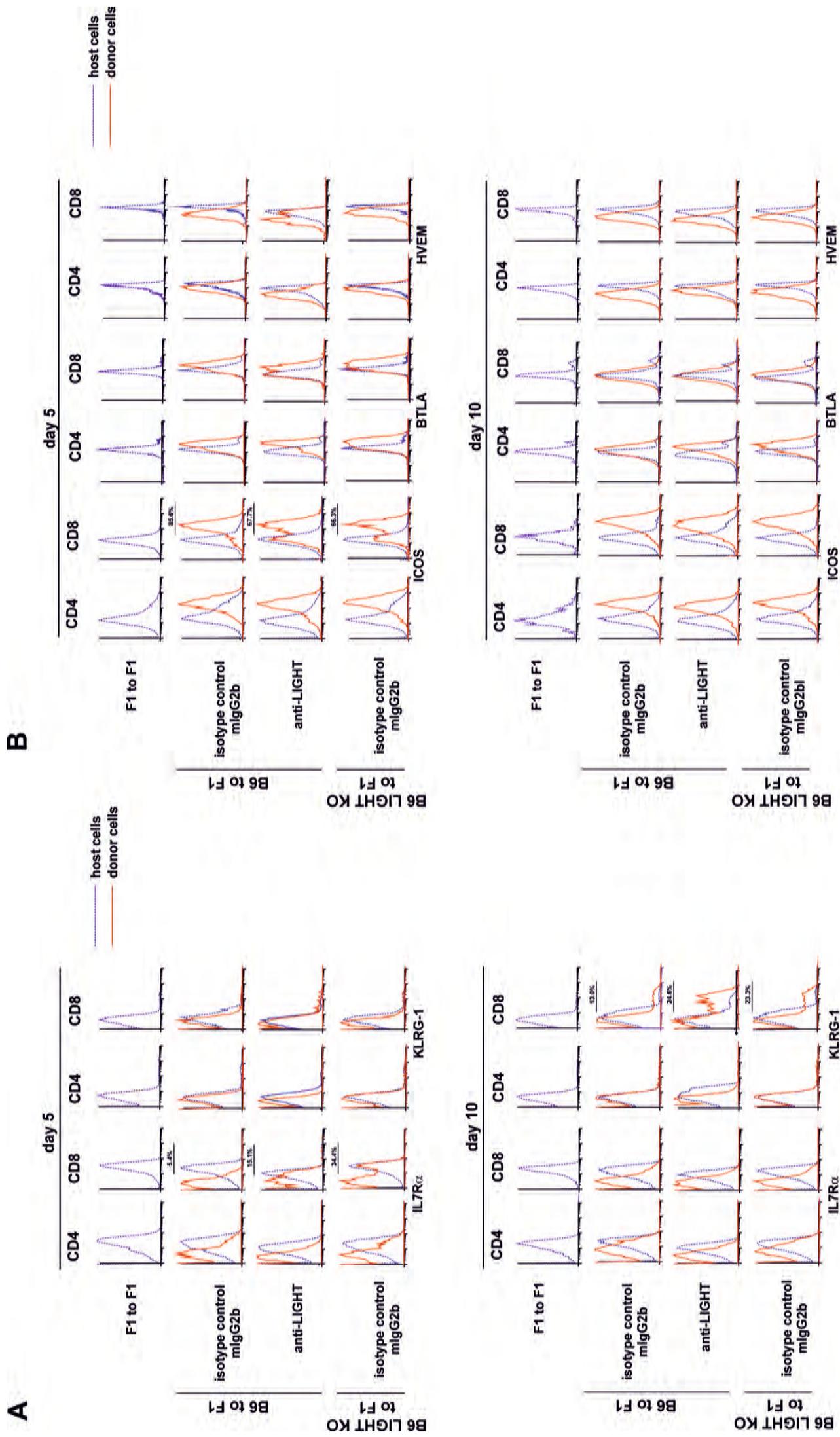
Figure 1

Figure 2

Figure 3

gated on donor CD4⁺ cells

gated on donor CD8⁺ cells

B6 to F1

B6 LIGHT KO to F1

% PF: 4.4
PI: 2.2

% PF: 4.6
PI: 2.1

isotype control mIgG2b

% PF: 2.3
PI: 1.5

% PF: 3.0
PI: 1.7

anti-LIGHT (3D11)

% PF: 2.9
PI: 1.5

% PF: 2.3
PI: 1.6

isotype control mIgG2b

cell count

CFSE

Figure 4

Figure 5