Dissociation of interferon-gamma production and resistance to leishmaniasis in the absence of tumor necrosis factor.

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Chapter 2 The role of TNF in parasitic diseases: Still more questions than answers.

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Declaration of Ethics

The research presented in this thesis was conducted within the guidelines of the James Cook University Statement and Guidelines on Research Practices which is based on the NHMRC Australian Code for the Responsible Conduct of Research (2007). The proposed research methodology received approval from the James Cook University Animal Ethics Committee (A1170 and A1492).

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Abbreviations

AICD	Activation induced cell death
B6	C57BL/6
CBA	Cytometric bead array
cDC	Conventional dendritic cell
ECM	Experimental Cerebral Malaria
eGFP	Enhanced Green Fluorescent Protein
ELISA	Enzyme linked immunosorbent assay
FoxP3	Forkhead box P3
Gata3	GATA binding protein 3
H2	Histocompatability 2
IFNγ	Interferon gamma
IL-4	Interleukin 4 protein
Il-4	Interleukin 4 gene-
IL-10	Interleukin 10 protein
Il10	Interleukin -10 gene

IL-17A	Interleukin 17 A protein
Il-17A	Interleukin 17 A gene
iNOS	Inducible Nitric Oxide Synthase
kDa	KiloDalton
LACK	*Leishmania* homolog of receptor for activated c- kinase
LN	Lymph node
LTα	Lymphotoxin alpha
LTβ	Lymphotoxin beta
MHC	Major Histocompatability Complex
Mo-DC	Monocyte derived Dendritic Cell
NFκB	nuclear factor of kappa light polypeptide gene enhancer in B-cells
NO	Nitric Oxide
OVA	Ovalbumin
PBS	Phosphate Buffered Saline
pLN	Popliteal lymph node

Rorc	See RorT
RorγT	retinoic acid receptor-related orphan receptor gamma
Tbet	See Tbx21
Tbx21	T box family of transcription factors
TCR	T cell receptor
Th1	T helper 1
Th2	T helper 2
TIM	TNF receptor-associated factor interacting motifs
Tnf	Tumor necrosis factor - gene
TNF	Tumor necrosis factor - protein
TNFR1	TNF receptor 1 (p55,p60)
TNFR2	TNF receptor 2 (p75, p80)

Abstract

The delineation of T helper 1(Th1) and T helper 2 (Th2) responses in promoting resistance and susceptibility to experimental cutaneous leishmaniasis has provided a substantial contribution to the understanding of the molecular basis of T cell differentiation in the context of infectious disease. Dysregulation of these processes renders the host susceptible to disease pathogenesis or immuno-pathology. Yet, the paradigm of resistance and susceptibility fails if the adaptive immune systems is not coupled adequately to the innate immune system. The pleiotropic cytokine Tumor necrosis factor (TNF) is involved in numerous aspects of homeostatic and inflammatory processes involved with immune cell function. Dysregulation of TNF production is associated with autoimmune diseases such as Rheumatoid Arthritis, or can render the host susceptible to infectious diseases. The mechanisms however, by which the overproduction of, or the lack of TNF promotes these extreme outcomes is still relatively unknown. Here, I analsysed the genetic contribution of the different major components of the TNF signalling family to elucidate how TNF confers protection to infection with the intracellular protozoan parasite *Leishmania major*.

Co-operative induction of inducible nitric oxide synthase (iNOS) in mononuclear phagocytes by Interferon gamma and TNF provides the basis for an effective immune response to *L. major*. In the absence of TNF the normally resistant C57BL/6 mouse strain develops a fatal visceralising form of leishmaniasis. Protection from this fatal outcome is dependent on the expression of the trans-membrane but not the soluble form of TNF through an interaction with TNFR1, however the mechanism by which this interaction confers protection remains unknown.

Here I demonstrate that this susceptibility to infection does not result from altered $CD4^+$ effector T cell differentiation or inpaired induction of iNOS. T cell activation is greatly increased in the absence of TNF, however enhancement of activation as measured by increased CD44 expression does not reflect positively on the clinical outcome. $CD44^+$ $CD4^+$ T cells from *L. major* infected TNF-deficient mice showed similar transcriptional up-regulation of both *Tbx-21* and *Ifn-γ* compared to WT controls but showed reduced expression of both *Gata-3* and *Il-10* indicating a more polarized T cell response. This was similarly accompanied by increased levels of IFN-γ that was observed locally and systemically in the absence of either TNF or TNFR1. The up-regulation of IFN-γ in both resistant B6.WT and susceptible B6.TNF-deficient mouse strains correlated with the induction of iNOS that was predominantly expressed by infiltrating $CCR2^+$ inflammatory monocytes. Despite equivalent induction of iNOS in both the lesion and draining lymph node, expression of iNOS and location of *L. major* amastigotes showed distinct cellular compartmentalization. While iNOS expression was restricted to $CCR2^+$ inflammatory monocytes, a novel $CD11b^+$, $iNOS^-$, $Ly6G^-$, $Ly6C^{low}$, $CCR2^{low}$ population was observed that was highly parasitised and accumulated exclusively in the absence of either TNF or TNFR1 in the draining lymph node. The capacity for these $CD11b^+$, $iNOS^-$, ,$Ly6G^-$, $Ly6C^{low}$, $CCR2^{low}$ cells to become highly parasitised did not result from any intrinsic deficit of TNFR signalling. Rather, mixed bone marrow chimeras showed that this sensitivity to *L. major* parasitism results from external cues generated upstream of monocyte and macrophage activation that renders these cells susceptible to infection.

These data demonstrate a unique role for TNF in the coupling of innate and adaptive immune responses through modulating the development of infiltrating myeloid cells that have different leishmanicidal potentials and reflect a state of susceptibility to intracellular

infection to *L. major* rather than promoting direct leishmanicidal functions *in vivo*.

Manuscripts and Presentations Arising From This Thesis.

Korner, H., McMorran, B., Schluter, D., and Fromm, P. (2010). The role of TNF in parasitic diseases: Still more questions than answers. Int J Parasitol *40*, 879-888.

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Phillip Fromm, Heinrich Körner. Dissociation of Interferon gamma and resistance to murine Leishmaniasis in the absence of TNF. Brisbane Immunology Group Annual Conference; 2010 Invited Session talk.

Phillip Fromm, Heinrich Körner Fatal Leishmaniasis despite iNOS production in the absence of TNF. Australasian Society of Immunology Annual conference 2009 - Immune Responses to Infectious Diseases -Workshop talk.

Alicia Roomberg, **Phillip Fromm**, Florian Wiede, Heinrich Körner. CCR6 modifies germinal centre reaction and secretion of immunoglobulin through modulation of follicular T helper cell activation. - Brisbane Immunology Group Annual Conference - Post Graduate Talk- 2009

Phillip Fromm, Christian Bogdan, Heinrich Körner. Up-regulation of IL-6 in TNF-deficient mice correlates with a fatal outcome of Leishmania major despite a strong IFNγ response. - Australian Society of Immunology Annual Conference 2009 - Immunological Challenges in the 21st Century - Poster Presentation

Phillip Fromm, Heinrich Körner. Infection of TNF-deficient mice with *L. majo*r results in dysregulated cytokine expression and a skewed Regulatory T cell response. - Brisbane Immunology Group Annual Conference – Poster Presentation 2008.

Phillip Fromm, Heinrich Körner. TNF-deficient mice exhibit significant changes in the cell-mediated immune response to *L. major*. -Brisbane Immunology Group Annual Conference- Post Graduate Talk- 2007.

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