A transgenic mouse model approach to investigate the interactions between T cells during the course of an immune response

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Preface

The work described in this thesis was designed to investigate the interactions between T cells during an immune response through the use of a number of different strains of T cell receptor transgenic mice

All experiments were carried out by the author at the Centenary Institute of Cancer Medicine and Cell Biology between February 2001 and April 2006. Approval for animal experimentation was obtained from Institutional Ethics Committee at the University of Sydney. The work is entirely original and has not been presented previously for purpose of obtaining another degree.

Recombinant HELMCC-his protein used in all experiments described in Chapter 4 and in the experiments described in Figure 5.7.1 and Figure 5.7.2 was produced by Didrik Paus (B Cell Biology Group).

Publication arising from work presented in this thesis

Alexandra J Spencer, Didrik Paus, Adrian L. Smith, Robert Brink, Barbara Fazekas de St Groth (2006). Antigen-specific competitive inhibition of $CD4^+$ T cell recruitment into the primary immune response (submitted)

Alexandra J. Spencer and Barbara Fazekas de St Groth (2006). The effect of competition on the generation of effector and memory cells (manuscript in preparation)

Alexandra J. Spencer, Ben Roediger and Barbara Fazekas de St Groth (2006). A phenotypic characterisation of DCs during the course of an adjuvant-enhanced CD4 response. (manuscript in preparation)

Abstract

The experiments described in this thesis document the development of two *in vivo* models, to investigate the effect of competition for peptide-MHC and factors independent of MHC on T cell proliferation, differentiation, generation of memory cells and affinity maturation.

The first model made use of 3 strains of T cell receptor (TCR) transgenic (tg) mice of varying specificity for antigen-MHC class II. To determine the effect of antigen specific and non-specific competition on the early stages of the T cell response, the efficiency with which naïve antigen-specific $CD4^+$ T cells were recruited into an ongoing immune response was investigated. Recruitment into cell division and cytokine production was shown to decrease with an increasing time delay between two cell cohorts of the same specificity, leading to a significant drop in recruitment with a delay of only 24 hours. Injection of additional antigen could partially compensate for this decrease, suggesting that lack of available antigen limited recruitment of specific cells trafficking to the node after the initiation of the response. A role for antigen non-specific factors such as access to APCs, costimulatory signals or cytokines was ruled out by showing that the response to a second, independent antigen was unaffected by an ongoing response, even when the same APCs were presenting both antigens.

The second system modelled a situation in which a clone of uniformly high affinity T cells competed against a polyclonal population containing mixture of affinities. This situation would arise during a normal response to a single epitope, and would mimic the process of competition that drives affinity maturation of the $CD4^+$ T cell response. By substituting a high affinity response to a different antigen, a more complex reaction to multiple antigens, of different affinities was modelled. To avoid any possible effect of the two antigens competing for access to processing machinery, or binding to the same MHC class II allele, the two antigens were provided as synthetic peptides that bind to different MHC molecules. The data indicated that $CD4^+$ T cell competition for peptide-MHC is far more

potent than competition between $CD4^+$ T cell responses of different specificity. Antigen-specific competition reduced the level of T cell stimulation detected as early as day 3 of the response. In the face of high affinity antigen-specific competition, the representation of mixed affinity T cells within the effector and effector memory cells (T_{EM}) population declined progressively throughout the primary and secondary responses, suggesting that continued access to peptide-MHC is required to maintain maximum numbers of effector and T_{EM} cells. In contrast, the contribution of central memory (T_{CM}) was stable from day 7 onwards. Competition by $CD4^+$ cells of an unrelated antigenic specificity led to a minor reduction in peak cell number and cytokine production in the primary response, without altering the number or potency of memory cells.

Together these two models demonstrated a mechanism whereby the immune system exerts tight control over the size and kinetics of each individual antigen specific response without affecting the ability to respond to secondary infections or late-phase lytic antigens. Overall the results demonstrate a continued requirement for TCR stimulation for the generation of effector cells and the maintenance of a population of cytokine producing memory cells. However the generation of a stable population of central memory cells was unaffected by conditions of reduced T cell stimulation, ensuring that long-term memory can be maintained in the absence of antigen.

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Abbreviations

Ag	antigen
AICD	activation induced cell death
APC	antigen presenting cell
B7RP-1	B7 related protein
BrdU	5-bromo-2'deoxyuridine
BM	bone marrow
BSA	bovine serum albumin
BTLA	B and T lymphocyte attenuator
C	constant
Ca^{2+}	calcium
CD	cluster of differentiation
CDR	complementarity determining region
CHO	chinese hamster ovarian
CFA	complete Freuds adjuvant
CFSE	carboxyfluorescein diacetate succinimidyl ester
CRD	cysteine rich domain
CrmA	cytokine response modifier A
Cyt-C	Cytochrome C
CTLA-4	cytotoxic T lymphocyte antigen-4
D	diversity
DC	dendritic cell
DAPI	4',6'-diamidino-2-phenylinodole dihydrochloride
ELISA	enzyme-linked immunosorbent assay
FADD	Fas associated death domain
FCS	foetal calf serum
FLICE	Fas-associated death domain-like IL-1β converting enzyme
FLIP	FLICE inhibitory protein
FW	facswash
GM-CSF	granulocyte/macrophage colony stimulating factor
HEL	hen egg lysozyme

^{hi}	high
ICAM	intracellular adhesion molecule-1
ICOS	inducible costimulator
IFN	interferon
Ig	Immunoglobulin
IL	interleukin
^{int}	intermediate
i.p.	intraperitoneal
IRAK	IL-1R associated kinase
i.v.	intravenous
J	joining
KO	knockout
LB	liquid broth
LC	Langerhans cells
Lck	$p56^{lck}$
LCMV	lymphocytic choriomeningitis virus
LFA-1	lymphocyte function-associated antigen-1
LN	lymph node
^{lo}	low
LPS	lipopolysaccharide
LRR	leucine rich repeat
mAb	monoclonal antibody
MBP	myelin basic protein
MCC	tobacco hornworm moth cytochrome C
MHC	major histocompatibility complex
NPP	nitrophenyl phosphate
Ova	ovalbumin
PAMP	pathogen-associated molecular pattern
PBS	phosphate buffered saline
PCC	pigeon cytochrome c
PCR	polymerase chain reaction
PD-1	programmed cell death

PFA	paraformaldehye
PI	propidium iodide
PLP	proteolipid protein
PRR	pattern recognition receptor
RPMI	Roswell Park Memorial Institute (medium)
s.c.	sub-cutaneous
SPF	specific pathogen free
TBS	tris buffered saline
Tc	cytotoxic T cell
T_{CM}	T central memory
TCR	T cell receptor
T_{EM}	T effector memory
Tg	transgenic
T_h	helper T cell
TIR	Toll/IL-1R
TLR	toll-like receptor
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
V	variable
μg	micrograms