THE UNIVERSITY OF MANITOBA

ROLE OF SIX HOUR T CELLS IN IMMUNITY

by

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TO MY WIFE, SELINA AND MY PARENTS

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RATIONALE

Ever since the discovery that the collaboration of two lymphocyte populations were necessary for the development of humoral antibody responses, data have rapidly accumulated to implicate participation of T cells in many immune phenomena. It also soon became evident that T cells play a dual role in the development of immune responses. On the one hand, T cells have been shown to provide critical 'helper' effects, while on the other hand, T cells have been equally well known to exert negative effects on the development of immunity. These observations generated a number of significant and interesting questions. For example, what are the natures of the helper and inhibitory cells? Do they represent different subpopulations of T cells or are they one cell at different stages of differentiation? What is the mechanism(s) underlying collaboration and initiation of an immune response? Once induced, how is an immune response regulated and terminated?

Previous studies in this laboratory demonstrated that a relatively large subpopulation (25%) of splenic T cells acquired readily detectable surface immunoglobulin within 6 hours following antigenic stimulation (1,2). Complexes of Ig and antigen are present in serum at the same time and have been shown to be cytophilic for T cells in vitro (3). This observation, which represents one of the earliest known phenomena induced in vivo by antigenic stimulation, posed the immediate question as to its functional relevance. Basically, two approaches were adopted to delineate the biological

significance of the observed changes at 6 hours. A direct approach involving the adoptive transfer of 6 hour cells has been undertaken by others in the laboratory.

A different approach is to abrogate the immune response by some tolerizing regime and then observe the effects on the 6 hour phenomenon.

The subject of this thesis represents efforts along the lines of the second approach in an attempt to answer the following questions:

(1) Does the 6 hour phenomenon have a role in humoral antibody formation or cell mediated immunity or both? If so,

(2) does it reflect an inductive or regulatory mechanism?

It is hoped that the results of these efforts will throw some light on the fundamental issues of induction, regulation and termination of the immune response and contribute to a better understanding of the complexities of the immune system.

In addition, preliminary experiments to define the role of suppressive factors in this mode of tolerance induction are also included.

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ABC	antigen binding cell
Ab(s)	antibody (ies)
ALS	anti-lymphocyte serum
$\alpha\theta$	anti-theta antiserum
αMIg-αBSA	hybrid antibody which detects all classes of mouse Ig.
ATS	anti-thymocyte serum
B cells	bone marrow derived lymphocytes
BCG	Bacillus Calmette-Guerin
BDB	Bis-diazotized benzidine
BSA	bovine serum albumin
CMC	cell-mediated cytotoxicity
CNBr	cyanogen bromide
Con A	concanavalin A
CRBC	chicken red blood cells
CRL	complement receptor carrying lymphocytes
DEAE	diethylaminoethyl
DTH	delayed type hypersensitivity
EA	chicken egg albumin
FCA	Freund's complete adjuvant
FCS	fetal calf serum
Fe	ferritin
FIB	human fibrinogen
HBSS	Hank's balanced salt solution

HHS	horse erythrocyte hemolysate supernatant
HRBC	horse red blood cells
Ig	immunoglobulin
i.p.	intraperitoneally
KLH	keyhole limpet hemocyanin
MBLA	mouse specific bone marrow derived lymphocyte antigen
MHC	major histocompatibility complex
MSLA	mouse specific lymphocyte antigen
NRS	normal rabbit serum
PFC	plaque forming cell
PHA	phytohemagglutinin
POL	polymerized flagellin
RFC	rosette forming cell
RICA	reverse immunocytoadherence
R.T.	room temperature
SIII	pneumococcal polysaccharide type III
SHS	sheep erythrocyte hemolysate supernatant
SRBC	sheep red blood cells
T cells	thymus derived lymphocytes
T-RFC	T-rosette forming cell
θ	theta isoantigen

ABSTRACT

Investigations have been undertaken in our laboratory to study the early effects of antigen on B and T cells in spleens of mice during a primary response to antigen. The reverse immunocytoadherence technique (RICA) was used to detect surface associated immunoglobulin on mouse lymphocytes. The technique involves the use of a 5s hybrid antibody which has an anti-mouse Ig site and an antiprotein site. A rosette is formed as a result of the reaction between the anti-Ig site with surface associated Ig and the antiprotein site with protein coated sheep red cells. Utilizing RICA and an antitheta antiserum-complement cytotoxicity assay, it has been demonstrated that 25% of splenic T cells acquired easily demonstrable surface Ig 6 hours after primary immunization. Concomitantly, there is a comparable decrease in the level of theta carrying lymphocytes. Furthermore, cytophilic complexes of antigen with immunoglobulin mediated by a soluble factor have also been detected in sera of immune animals.

This thesis studies the effects of antigen upon B and T cells in animals rendered immunologically unresponsive to HRBC, and evaluates the effects of tolerance upon the serum factors and cytophilic complexes identified during a normal primary response.

Mice, made unresponsive to HRBC by repeated injections of an ultracentrifuged horse red blood cell hemolysate preparation, were challenged with an immunogenic dose of HRBC

and their spleen cells analyzed 6 hours after challenge. In contrast to normal mice, T cells in these animals did not acquire surface immunoglobulin as detected by RICA. Similarly no 6 hour Ig carrying T cells were observed upon immunizing tolerant mice with a battery of heterologous antigens. Experiments further revealed that tolerant spleen cells were unable to pick up preformed homologous or heterologous cytophilic complexes reflecting some deficit in their membrane properties. However, six hour sera collected from tolerant mice immunized with particulate antigens only contained cytophilic complexes. Whether these complexes are quantitatively and qualitatively similar to those obtained from normal mice is uncertain at the present time. Although the 6 hour T cells appear not to be directly involved in the specific induction of humoral responses, it is quite possible that they may be concerned with the induction of other aspects of the immune response and/or with immunoregulation. Results of experiments reported here suggested that the 6 hour T cell may well play some role in regulating T cell mediated immune functions such as delayed type hypersensitive reactions, cytotoxic effector lymphocyte activity and DNA synthetic responses to plant mitogens. Other results implicate the 6 hour T cell in an amplifying role in antibody formation. Thus, 6 hour immune spleen cells obtained from normal animals immunized with SRBC were capable of specifically augmenting the antibody response to SRBC in an irradiated recipient. On the other hand, six hour spleen cells from SRBC immunized

HRBC tolerant animals, which have been shown to lack Ig+ T cells failed to show any enhancing capabilities. Nevertheless, they were fully capable of supporting a normal anti SRBC response comparable to that given by nonprimed normal spleen cells. These findings strongly suggest that the 6 hour T cells and the more conventionally known Ig- 'helper T cells' are two distinct subpopulation of primed T cells. The hypothesis is proposed according to which the 6 hour T cell probably subserves a regulatory role and amplifies the basic helper T function by some as yet undetermined mechanism.

The possible role of suppressive factors mediating this particular type of tolerance was also investigated. Preliminary data, although suggestive of the presence of suppressive activity in tolerant serum are far from conclusive and have not established any strict correlation between these suppressive factors and tolerance induction or maintainance.

LITERATURE REVIEW