

THE UNIVERSITY OF MANITOBA

ROLE OF SIX HOUR T CELLS IN IMMUNITY

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF IMMUNOLOGY
FACULTY OF MEDICINE

WINNIPEG, MANITOBA

May 1978

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A dissertation submitted to the Faculty of Graduate Studies of
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TO MY WIFE, SELINA AND MY PARENTS

ACKNOWLEDGEMENTS

The author would like to express his sincerest gratitude to his supervisor, Dr. F. Paraskevas for his invaluable supervision, advice and guidance throughout the years of association and in the preparation of this thesis.

Appreciation is also extended to Dr. S.T. Lee, Dr. K.B. Orr and Dr. D.A. Chow for their helpful suggestions and enlightening discussions during the years of working together.

Thanks are also extended to the technical staff in Dr. Paraskevas' laboratory for their excellent assistance.

The financial supports of the University of Manitoba, the Medical Research Council, and the National Cancer Institute are gratefully acknowledged.

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.

TABLE OF CONTENTS

	Page
LITERATURE REVIEW	
I. IMMUNOLOGICAL CONCEPTS: A BRIEF HISTORY.....	1
II. CELLULAR COMPONENTS OF THE IMMUNE SYSTEM	6
1. Hemapoietic Stem Cells	6
2. Primary Lymphoid Organs	6
i. The Thymus	7
ii. The Bursa of Fabricius and Possible Mammalian Analogs	8
3. Secondary Lymphoid Organs	9
4. Immunocompetent Cell	10
i. Functional Characteristics	10
A. Thymus Derived Lymphocytes	11
B. Bone Marrow Derived Lymphocytes	12
C. Accessory Cells	14
ii. Structural Characteristics	14
A. Bone Marrow Derived Cells	14
(a) Surface Ig	14
(b) Complement Receptors	18
(c) Fc Receptors	19
(d) Mouse Specific Bone Marrow Derived Lymphocyte Antigen (MBLA)	20
B. Thymus Derived Cells	20
(a) Surface Ig	20
(b) Fc Receptors	22
(c) Theta-alloantigen	22
(d) Mouse Specific Lymphocyte Antigen (MSLA)	23

	Page
III. IMMUNE RECOGNITION	23
1. Antigen Binding Cells	24
i. Rosette Formation	24
ii. Autoradiographic and Fluorescent Techniques	25
iii. Enzymatic Methods	25
iv. Solid Phase Methods	26
2. Antigen Receptors	30
i. Nature of Ag Receptor	30
A. Ability of Anti-Ig to Induce Trans- formation and Mitosis of Treated Lymphoid Cells	30
B. Anti-Ig Inhibition of Antigen Binding Cells	31
(a) Inhibition of "B" Cell ABC	31
(b) Inhibition of T Cell ABC	32
C. Anti-Ig Inhibition of B and T Cell Functions	34
3. Mechanism of Binding	40
IV. CELL INTERACTIONS	42
1. Humoral Immunity	42
2. Immunologic Memory	45
3. Carrier Effect	47
4. Antigenic Competition	49
5. Cell-Mediated Immunity	50
6. Hypothesis of Mechanisms of Co-operation	52
i. Focusing Theory	53
ii. Minimal Model	54

	Page
iii. Major Histocompatibility Complex Control of T-B Collaboration	55
iv. T Cell Mediator Model	57
V. REGULATION OF IMMUNE RESPONSES	59
1. Enhancement	60
i. Specific T Cell Influence	60
ii. Antibody Mediated	61
iii. Amplifier T Cell	62
iv. Non-specific T Cell Influence	63
2. Suppression:T Cell Suppressive Influence	63
i. Tolerance Induction	64
ii. Immune Responses to T-independent Antigen.	65
iii. Antigenic Competition	66
iv. Autoimmunity	67
v. Tumor Immunology	67
vi. Genetically Determined Immunologic Unresponsiveness	69
vii. Cell-mediated Immunity	69
VI. IMMUNOLOGIC TOLERANCE	71
1. Sites of Immunological Unresponsiveness	71
2. Tolerance to Selected Antigens	73
i. Heterologous Serum Proteins	73
ii. Antigens with Multiple Repeating Determinants	76
iii. SRBC	78
3. Antibody Mediated Suppression	80
4. Mechanism of Tolerance Induction	82

	Page
MATERIALS AND METHODS	
I. MICE	85
II. RABBITS	85
III. HORSE AND SHEEP ERYTHROCYTE HEMOLYSATE SUPERNATANT..	85
IV. ANTIGENS.....	86
1. Mouse IgG and IgF Myeloma Proteins	86
2. Horse Spleen Ferritin	87
3. Bovine Serum Albumin and Chicken Egg Albumin ...	87
4. Human Fibrinogen	87
5. Chicken Erythrocytes	87
6. Horse and Sheep Erythrocytes	88
V. ADJUVANTS	88
VI. RABBIT IMMUNIZATION AND COLLECTION OF ANTISERA	88
VII. MOUSE IMMUNIZATION AND COLLECTION OF SERA	89
VIII. ION EXCHANGE CHROMATOGRAPHY BY DEAE CELLULOSE	89
IX. SEPHADEX G100 AND G200 GEL FILTRATION	91
X. IMMUNOELECTROPHORESIS	92
XI. OUCHTERLONY GEL DIFFUSION	93
XII. IMMUNOADSORBENTS	93
1. Bis-diazotized Benzidine Method	93
2. Ethyl Chloroformate Method	95
3. Cyanogen-bromide Activated Sepharose 4B Method..	95
XIII. PURIFICATION OF ANTIBODIES BY IMMUNOADSORBENTS	96
1. By Antigen Aggregates	96
2. By Conjugated Sepharose	97
XIV. PEPSIN DIGESTION OF ANTIBODIES	97

	Page
XV. COATING OF SHEEP RED BLOOD CELLS	98
XVI. HYBRID ANTIBODY PREPARATION	99
XVII. REVERSE IMMUNOCYTOADHERENCE (RICA) TECHNIQUE	100
1. Preparation of Single Cell Suspensions	101
2. RICA Assay	101
XVIII. ENUMERATION OF THETA ANTIGEN POSITIVE T LYMPHOCYTES BY CYTOTOXICITY TEST	102
1. Preparation of Anti-theta Antiserum	102
2. Anti-theta Cytotoxicity Test	103
XIX. INDUCTION OF IMMUNOLOGIC UNRESPONSIVENESS TO HRBC OR SRBC	103
XX. PLAQUE FORMING CELL ASSAY	104
XXI. PHYTOHEMAGGLUTININ STIMULATED LYMPHOCYTE TRANS- FORMATION: TRITIATED THYMIDINE UPTAKE	105
XXII. CELL MEDIATED CYTOTOXICITY ASSAY	106
1. Labelling of Target Cells with ⁵¹ Cr	107
2. Preparation of Effector Spleen Cells	107
3. CMC Assay	107
XXIII. DELAYED TYPE HYPERSENSITIVITY	108
1. Sensitization	108
2. Footpad Assay of DTH	109
XXIV. PASSIVE HEMAGGLUTINATION TEST	110
XXV. IRRADIATION AND ADOPTIVE TRANSFER OF SPLEEN CELLS..	110
XXVI. SPLEEN CELL SUSPENSIONS	110
1. Normal Adult Balb/c Mice	110
2. HRBC Tolerant Adult Balb/c Mice	112

	Page
XXVII. TREATMENT OF NORMAL SPLEEN CELLS IN VITRO	114
XXVIII. TREATMENT OF TOLERANT SPLEEN CELLS IN VITRO	115
XXIX. IN VITRO PREPARATION OF IACF	115
XXX. PREPARATION OF SERUM UM10 ULTRAFILTRATES	115a
XXXI. STATISTICAL ANALYSIS	115a

RESULTS

I. Ig BEARING LYMPHOCYTES IN SPLEENS OF NORMAL ADULT BALB/C MICE	116
II. THETA (θ) BEARING LYMPHOCYTES IN SPLEENS OF NORMAL ADULT BALB/MICE	117
III. TOTAL NUMBER OF SPLEEN CELLS IN NORMAL ADULT BALB/C MICE	117
IV. Ig CARRYING LYMPHOCYTES IN SPLEENS OF BALB/C MICE 6 HOURS AFTER IMMUNIZATION WITH ANTIGEN	117
V. θ CARRYING LYMPHOCYTES IN SPLEENS OF BALB/C MICE 6 HOURS AFTER ADMINISTRATION OF ANTIGEN	118
VI. TOTAL SPLEEN CELLS IN IMMUNE ADULT BALB/C MICE ...	121
VII. NORMAL ANTI-HRBC PFC RESPONSE	123
VIII. SUPPRESSION OF HRBC PFC RESPONSE WITH MULTIPLE INJECTIONS OF HORSE ERYTHROCYTE HEMOLYSATE SUPERNATANT (HHS)	123
IX. SPECIFICITY OF SUPPRESSION	123
X. DURATION OF UNRESPONSIVENESS	126
XI. CHANGES IN Ig+ AND θ + LYMPHOCYTES IN THE SPLEENS OF NORMAL MICE DURING THE PRIMARY RESPONSE TO HRBC ..	132
XII. EFFECT OF PRETREATMENT WITH VARYING NUMBERS OF HRBC HEMOLYSATE SUPERNATANT INJECTIONS UPON THE SUBSEQUENT 6 HOUR RESPONSE TO IMMUNOGENIC HRBC STIMULATION	132
XIII. CHANGES IN Ig+ AND θ + SPLEEN CELLS IN TOLERANT MICE FOLLOWING IMMUNIZATION WITH HRBC	134
XIV. DURATION OF HRBC HEMOLYSATE SUPERNATANT INDUCED INABILITY TO PRODUCE 6 HOUR RESPONSE	137

	Page
XV. Ig+ CARRYING SPLEEN CELLS IN TOLERANT MICE SIX HOURS AFTER STIMULATION WITH HETEROLOGOUS ANTIGEN.	140
XVI. ABILITY OF TOLERANT MICE TO PRODUCE CYTOPHILIC COMPLEXES UPON STIMULATION WITH HETEROLOGOUS ANTIGENS	142
XVII. UPTAKE OF CYTOPHILIC COMPLEXES BY TOLERANT SPLEEN CELLS	142
XVIII. EFFECT OF TOLERANT SERUM UPON UPTAKE OF CYTOPHILIC COMPLEXES BY NORMAL SPLEEN CELLS	145
XIX. RESPONSE OF TOLERANT AND NORMAL SPLEEN CELLS TO PHYTOHEMAGGLUTININ STIMULATION	145
XX. CYTOTOXIC EFFECTOR CELL FUNCTION IN TOLERANT AND NORMAL SPLEENS	147
XXI. DELAYED TYPE HYPERSENSITIVITY REACTIONS IN NORMAL AND TOLERANT MICE	149
XXII. SPECIFIC ENHANCEMENT OF ANTIBODY PRODUCTION BY SIX HOUR SPLEEN CELLS	152
XXIII. EFFECT OF HRBC HEMOLYSATE SUPERNATANT PRETREATMENT ON THE ENHANCING ABILITIES OF SIX HOUR SPLEEN CELLS	154
XXIV. PARTIAL RESTORATION OF ANTI-HRBC RESPONSIVENESS IN HRBC TOLERANT MICE BY IACF FACTOR ADMINISTRATION	158
XXV. PRESENCE OF A SUPPRESSIVE ENVIRONMENT IN TOLERANT MICE	159
XXVI. DOES TOLERANT SERUM CONTAIN SUPPRESSIVE ACTIVITY?.	162
XXVII. LOSS OF SURFACE Ig INDUCED BY A SOLUBLE FACTOR PRESENT IN 7 DAY SERUM	162
XXVIII. SUPPRESSIVE ACTIVITY OF 6 DAY SERUM FROM NORMAL AND HRBC TOLERANT MICE IMMUNIZED WITH HRBC	163
DISCUSSION	172
BIBLIOGRAPHY	190

LIST OF TABLES

		Page
Table	I. Ig carrying spleen cells six hours after injection of antigen	119
Table	II. In vitro incubation of normal spleen cells with six hour serum elicited by different antigens	120
Table	III. Percent θ carrying lymphocytes in spleens of mice six hours after administration of antigen	122
Table	IV. Induction of unresponsiveness to HRBC	125
Table	V. Specificity of hemolysate supernatant induced tolerance: response of HRBC tolerant animals to SRBC and CRBC	127
Table	VI. Duration of HRBC hemolysate supernatant induced unresponsiveness	131
Table	VII. Effect of pre-treatment with varying injections of hemolysate supernatant upon the 6 hour response	135
Table	VIIIa. Changes in Ig+ spleen cells in mice pretreated with hemolysate supernatant ...	138
Table	VIIIb. Response of hemolysate supernatant pretreated mice 6 hours after immunogen administration given at various times after treatment	139
Table	IX. Ig+ spleen cells in tolerant mice six hours after heterologous antigen challenge	141
Table	X. Ability of tolerant spleen cells to produce cytophilic complexes upon challenge with heterologous antigen	143
Table	XI. Uptake of pre-formed cytophilic complexes by tolerant spleen cells	144
Table	XII. Effect of tolerant serum upon uptake of cytophilic complexes by normal spleen cells	146
Table	XIII. PHA reactivity of spleen cells from normal and tolerant mice	148

	Page
Table XIV. Cytotoxic effector cell function in normal and tolerant mice	150
Table XV. Delayed type hypersensitivity to HRBC, SRBC and ferritin in normal and tolerant mice	153
Table XVI. Specific enhancement of antibody production by six hour spleen cells	155
Table XVII. Lack of enhancing ability of 6 hour spleen cells from tolerant mice	157
Table XVIII. Effect of IACF factor on anti-HRBC response of tolerant mice	160
Table XIX. Presence of suppressive environment in tolerant mice	161
Table XX. Effect of tolerant serum upon anti-HRBC PFC response of normal mice	164
Table XXI. Effect of UM10 filtrate of tolerant serum upon anti-HRBC PFC response of normal mice	165
Table XXII. 7 day spleen cells and serum of normal and hemolysate supernatant treated mice immunized with HRBC, CRBC and fibrinogen..	166
Table XXIII. Anti-HRBC PFC response of donor animals from which sera was obtained for preparation of UM10 filtrates	169
Table XXIV. Effect of UM10 filtrate of 6 day serum from normal and HRBC hemolysate supernatant treated mice immunized with HRBC on 20×10^6 normal spleen cells in an irradiated host	170

LIST OF FIGURES

	Page
Figure 1. Primary anti-HRBC response	124
Figure 2. Anti-ferritin response in normal mice	128
Figure 3. Anti-egg albumin response in normal and tolerant mice	129
Figure 4. Anti-fibrinogen response in normal and tolerant mice	130
Figure 5. Changes in Ig ⁺ and θ ⁺ spleen cells in normal mice following HRBC stimulation	133
Figure 6. Changes in Ig ⁺ and θ ⁺ spleen cells in tolerant mice following HRBC stimulation...	136
Figure 7. Experimental protocol: testing for suppressiv e activity in 6 day immune sera collected from normal and tolerant mice ...	167

LIST OF ABBREVIATIONS

ABC	antigen binding cell
Ab(s)	antibody (ies)
ALS	anti-lymphocyte serum
$\alpha\theta$	anti-theta antiserum
α MiG- α BSA	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 immunocytadherence 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