

THE UNIVERSITY OF MANITOBA

SUPPRESSION OF THE IgE ANTIBODY RESPONSE
WITH MODIFIED AND SOLUBLE ANTIGEN

by

LIONEL G. FILION

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

DEPARTMENT OF IMMUNOLOGY

WINNIPEG, MANITOBA

JANUARY, 1980

SUPPRESSION OF THE IgE ANTIBODY RESPONSE
WITH MODIFIED AND SOLUBLE ANTIGEN

BY

LIONEL G. FILION

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

DOCTOR OF PHILOSOPHY

© 1980

Permission has been granted to the LIBRARY OF THE UNIVER-
SITY OF MANITOBA to lend or sell copies of this thesis, to
the NATIONAL LIBRARY OF CANADA to microfilm this
thesis and to lend or sell copies of the film, and UNIVERSITY
MICROFILMS to publish an abstract of this thesis.

The author reserves other publication rights, and neither the
thesis nor extensive extracts from it may be printed or other-
wise reproduced without the author's written permission.

TO THE LORD GOD

ACKNOWLEDGEMENTS

I am greatly indebted to Drs. W.Y. Lee and A.H. Sehon for their supervision and encouragement during this work and also for their help and criticism in the preparation of this thesis.

The helpful suggestions and discussions received from Dr. R. Schwenk during the course of this study are also greatly appreciated.

The excellent technical and cheerful assistance of Karen Yamada during certain phases of this study are also gratefully acknowledged. I would also like to thank all of my friends within the department who have helped me in their own special ways.

Last but not least, I wish to thank my loving wife for her encouragement during the course of this study and for her typing of the thesis.

ABSTRACT

With an aim to devise immunologically specific methods for the suppression of reaginic antibody responses, two systems have been successfully developed by the use of (i) isologous murine gamma globulins (MyG) as nonimmunogenic carriers for the synthesis of tolerogenic derivatives of protein antigen (ovalbumin, OA), (ii) soluble protein antigen (dog albumin, DA). Indeed, the results of this investigation demonstrated that treatment with conjugates of OA-MyG, of normal mice or of mice presensitized with DNP-OA to a state of immediate hypersensitivity, suppressed the ability of these mice to produce anti-OA IgE antibodies, and of IgE antibodies to the hapten, 2,4-dinitrophenyl (DNP), when the latter was presented to the immunological system with OA as a carrier. However, presentation of this hapten on a different carrier led to normal anti-DNP and anti-carrier IgE responses, suggesting that this type of tolerance was highly carrier-specific. Moreover, experimental results in this study indicated that this mode of immunosuppression was at least in part mediated by suppressor cells. It was shown also that transfer of spleen cells from tolerant mice into normal and primed syngeneic recipients resulted in the suppression of primary and secondary IgE responses of the respective recipients on immunization with a sensitizing dose of DNP-OA.

In a parallel study, soluble DA induced a specific, long-lasting unresponsive state in normal mice or in mice presensitized with DA in spite of 2 subsequent sensitizations with DA. This state was mediated, at least partially, by the dampening of T helper cell function since mice primed with DNP-OA were unable to form anti-DNP IgE antibodies after their treatment with soluble DA. Moreover, in an adoptive transfer system, T cells from treated animals were not capable of providing the necessary helper function to B cells from mice primed with DA in order to elicit

III

anti-DA, IgE response. In contrast, T cells from mice primed with DA provided helper function to B cells either from treated mice or mice primed with DA. It was also shown that antigen specific Ts cells were at least partially responsible for the unresponsive state. In addition, a factor extracted from the Ts cell, abrogated the anti-DA IgE response in an adoptive transfer system.

IV

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	I
ABSTRACT	II
LIST OF FIGURES	VIII
LIST OF TABLES	IX
LIST OF ABBREVIATIONS	XI

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION	1
LITERATURE REVIEW	4

CHAPTER 2

SUPPRESSION OF THE IgE ANTIBODY RESPONSE TO OVALBUMIN IN MICE WITH
A CONJUGATE OF OVALBUMIN AND ISOLOGOUS GAMMA-GLOBULINS

INTRODUCTION	34
MATERIALS AND METHODS	37
Antigens	37
Other reagents	37
Animals	38
Immunization	38
Measurement of serum IgE antibodies	39
Measurement of hemagglutinating antibodies	40
Adoptive cell transfers	41
Preparation of OA-M γ G conjugate	41
Isolation and characterization of OA-M γ G conjugate	42
1. Fractionation Studies	42
2. Immunodiffusion analysis	43
3. Immuno-electrophoresis	43
4. Determination of the ratio of OA to M γ G	43
5. The " <u>in vivo</u> " half-life of OA-M γ G conjugate	44

6. Determination of molecular size	45
7. Preparation of OA-OA and OA-R γ G	45
RESULTS	
a) Evidence for the preparation of OA-M γ G conjugate	46
b) Physicochemical characteristics of OA-M γ G conjugate	50
c) Rate of clearance of OA-M γ G in normal mice	54
d) Preparation of OA-OA and OA-R γ G conjugate	54
e) <u>In vivo</u> : Effects of OA-M γ G	58
1. The effect of OA-M γ G, OA-R γ G, and OA-OA conjugates on the primary response to DNP-OA	58
a) Effect of OA-R γ G	58
b) Effect of polymerized OA	60
c) Effect of OA-M γ G	63
2. Effect of OA-M γ G on the secondary IgE response to OA and DNP	65
3. Effect of the dose of OA-M γ G on the anti-OA IgE response	70
4. Specificity of immunosuppression with OA-M γ G	70
f) The cellular mechanism of suppression due to the OA-M γ G conjugate	75
1. Effect of OA-M γ G conjugate on helper cells	75
2. Attempt to break tolerance by transfer of normal or DNP-OA primed cells into mice tolerized with OA-M γ G	78
3. Transfer of OA-M γ G induced tolerance into normal mice	82
DISCUSSION	92
CHAPTER 3	
SPECIFIC SUPPRESSION OF REAGINIC AND HEMAGGLUTINATING ANTIBODY RESPONSE TO DOG ALBUMIN (DA) BY THE ADMINISTRATION OF SOLUBLE DA	
INTRODUCTION	98
MATERIALS AND METHODS	99

VI

Animals	99
Protein antigens and other reagents	99
Half-life studies of ^{125}I -DA	99
Immunization and measurements of immune responses	100
Assay for plaque forming cells	100
RESULTS	101
a) Induction and kinetics of anti-DA IgE antibody responses	101
b) Suppression of the anti-DA primary and secondary IgE and H.A. antibody responses	105
c) Clearance and distribution of soluble ^{125}I -DA in mice primed with DA	108
d) Specificity of immunosuppression with soluble DA	113
e) Determination of antigenic cross-reactivity between OA and DA	117
DISCUSSION	120

CHAPTER 4

CELLULAR MECHANISM(S) INVOLVED IN THE SUPPRESSION OF ANTI-DA IgE RESPONSE OF MICE TREATED WITH SOLUBLE DA

INTRODUCTION	125
MATERIALS AND METHODS	127
Animals	127
Protein antigens and other reagents	127
Immunization and measurements of immune responses	127
Cytotoxic killing of splenic lymphocytes	127
RESULTS	129
a) Suppression of anti-DA IgE and H.A. antibody responses by soluble DA	129
b) Elucidation of the cellular mechanism(s) involved in the suppression of the anti-DA IgE responses of mice treated with soluble DA	129

VII

1. Transfer of spleen cells from mice treated with soluble DA into mice either primed with DA or irradiated (650R)	124
2. Effect of T helper cell	133
3. Types of cells suppressed by soluble DA	133
4. Identification of Ts cells	137
c) Characteristics of the Ts cell induced by soluble DA	139
1. Kinetics of appearance of Ts cells	139
2. Specificity of the suppressor cells induced by soluble DA	142
d) Partial characterization of Ts cell factor	147
1. Extraction of a soluble factor from Ts cells	147
2. Molecular weight of Ts cell factor	149
DISCUSSION	153

CHAPTER 5

GENERAL DISCUSSION	159
CONCLUSION	164
REFERENCES	167

VIII

LIST OF FIGURES

1. Elution profile of OA-M γ G through a Sephadex G-200 column	47
2. Immunodiffusion pattern of OA-M γ G	48
3. Immuno-electrophoresis pattern of OA-M γ G	49
4. Elution profile of 125 I labelled OA-M γ G conjugate	51
5. Elution profile of OA-M γ G through a Sepharose -4-B column	53
6. Determination of the clearance rate of 125 I labelled OA-M γ G and OA in normal mice	55
7. The elution profile of polymerized OA on gel filtration through a Sephadex G-200 column	57
8. Effect of OA-M γ G on the primary anti-OA and anti-DNP IgE responses	66
9. Effect of OA-M γ G on the primary anti-OA and anti-DNP hemagglutinating antibody responses.	67
10. Effect of native OA on the primary anti-DNP and anti-OA responses	68
11. Specificity of treatment with OA-M γ G in mice primed with RNase	74
12. Kinetics of anti-DA IgE and H.A. antibody responses	104
13. Suppression of the primary anti-DA IgE response	106
14. Suppression of the primary anti-DA H.A. response	107
15. Suppression of the secondary and tertiary IgE response	109
16. Suppression of the secondary and tertiary H.A. antibody responses	110
17. Clearance of 125 I-DA	112
18. Suppression of the anti-DA IgE and H.A. antibody response by soluble DA	130
19. Suppression of the anti-DNP response: Demonstration of diminished helper function	134
20. Kinetics of appearance of the T suppressor cells	140
21. Determination of the molecular weight of Ts cell factor on a Sephadex G-100 column (2.5 x 100 cm)	150

LIST OF TABLES

I.	Distribution of ^{125}I -labelled OA-M γ G in various organs	56
II.	Effect of rabbit γ -OA on the primary anti-DNP and anti-OA responses.	59
III.	Effect of highly polymerized OA on the primary anti-DNP and anti-OA IgE responses.	61
IV.	Effect of moderately polymerized OA on the primary anti-DNP and anti-OA IgE responses	62
V.	Effect of pretreatment with OA and OA-M γ G on the primary anti-DNP and anti-OA IgE responses.	64
VI.	Effect of OA-M γ G on the ongoing anti-DNP and anti-OA responses	69
VII.	Effect of the dose of OA-M γ G on the anti-OA IgE response	71
VIII.	Specificity of immunosuppression by OA-M γ G conjugate	73
IX.	Effect of OA-M γ G on helper "T" cell activity	76
X.	Effect of normal spleen cells on IgE responses of tolerized mice.	79
XI.	Effect of DNP-OA primed spleen cells on IgE responses of tolerized mice	80
XII.	Effect of normal spleen cells on DNP-OA primed and tolerized mice.	83
XIII.	Effect of DNP-OA primed spleen cells on DNP-OA primed and tolerized mice	85
XIV.	Transfer of tolerance into normal mice with spleen cells of OA-M γ G and DNP-OA treated mice	88
XV.	Transfer of tolerance into normal mice with day 7 spleen cells of DNP-OA primed and tolerized mice.	89
XVI.	Failure to transfer tolerance into normal mice with day 14 spleen cells of DNP-OA primed and tolerized mice	91
XVII.	Effect of antigen dose on the production of IgE response in B ₆ D ₂ F ₁ mice.	102
XVIII.	Increase in secondary IgE response with increase in interval between first and second sensitization	103
XIX.	Suppression of anti-DA plaque-forming cells responses by soluble DA	111

XX.	Residual ¹²⁵ I-DA in different organs at day 12	114
XXI.	Specificity of soluble DA induced suppression in normal B ₆ D ₂ F ₁ mice	115
XXII.	Specificity of soluble DA induced suppression in mice primed with DA	116
XXIII.	Determination of B cell cross-reactivity between DA and OA	118
XXIV.	Failure to transfer suppression induced by soluble DA into mice primed with DA	131
XXV.	Maintenance of suppression induced by DA in adoptive transfer	132
XXVI.	Type of cells suppressed by soluble DA	135
XXVII.	Identification of suppressor T cells in mice treated with soluble DA	138
XXVIII.	Specificity of the suppressor cells induced by soluble DA	143
XXIX.	Suppression of helper function with suppressor T cells.	145
XXX.	Demonstration of suppressor T cell factor	148

ABBREVIATIONS

Al(OH) ₃	:	aluminum hydroxide
ALS	:	anti-mouse lymphocyte serum
anti-	:	antibody
α-A	:	α-amylase
Asc	:	<u>Ascaris suum</u> extract
B	:	bursa of Fabricius equivalent cell
B _e	:	bursa of Fabricius equivalent cell producing IgE
BBS	:	borate buffered saline
BGG	:	bovine gamma globulin
BPO	:	benzylpenicilloyl group
BSA	:	bovine serum albumin
B ₆ D ₂ F ₁	:	(C ₅₇ Bl/6 x DB A/2)F ₁ mice
CON A	:	concanavalin A
C'	:	complement
CPM	:	counts per minute
CY	:	cyclophosphamide
DA	:	dog albumin
DNP	:	dinitrophenyl group
D-GL	:	copolymer of D-glutamic acid -D- lysine
FCA	:	Freund's complete adjuvant
FCS	:	fetal calf serum
FIA	:	Freund's incomplete adjuvant
GAT	:	copolymer of L-glutamic acid, L-alanine, L-tyrosine
γ	:	gamma
H.A.	:	hemagglutination
H-2	:	major histocompatibility complex of the mouse
HSA	:	human serum albumin

XII

Ig	:	immunoglobulin
IgG	:	immunoglobulin of the G class
IgE	:	immunoglobulin of the E class
^{125}I	:	radioactive isotope of iodine, 125
i.p.	:	intraperitoneally
Irgene	:	immune response gene
I-Jgene:	:	gene located in the J region of the H-2 complex
i.v.	:	intravenously
KLH	:	keyhole limpet hemocyanin
MEM	:	minimal essential medium
2ME	:	2- mercaptoethanol
ml	:	milliliter
mg	:	milligram
MyG	:	mouse gamma globulin
MIgG	:	mouse immunoglobulin of the G class
MIgM	:	mouse immunoglobulin of the M class
MSA	:	mouse serum albumin
NMS	:	normal mouse serum
OA	:	ovalbumin
OA-MyG	:	ovalbumin conjugated to MyG
OA-RyG	:	ovalbumin conjugated to RyG
PCA	:	passive cutaneous anaphylaxis
PEG	:	polyethylene glycol
PFC	:	plaque forming cells
PBS	:	phosphate buffered saline
PVA	:	polyvinyl alcohol
RAG	:	water soluble extract of ragweed pollen

XIII

RYG	:	rabbit gamma globulin
RNase	:	ribonuclease
RPM	:	revolutions per minute
SRBC	:	sheep red blood cells
T	:	thymus derived cells
θ	:	theta antigen
Tbc	:	mycobacterium
Tca	:	trichloroacetic acid
Th	:	thymus derived helper cells
Ts	:	thymus derived suppressor cells
UD-C	:	urea denatured carrier
μ g	:	microgram
μ l	:	microliter

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

It has been well established that common forms of hypersensitivity of the immediate type are mediated primarily by IgE antibodies, produced by allergic individuals in response to a wide spectrum of allergens. In an attempt to devise effective treatments based on the current knowledge of the cellular interactions regulating the formation of antibodies in general (1) and of IgE antibodies in particular (2-5), many studies have been directed in recent years with a view to developing specific methods for the immunosuppression of IgE antibodies. Thus, the administration of conjugates, consisting of haptens covalently linked to nonimmunogenic molecules, specifically abrogated the IgE response to the respective antigen (reviewed in 6). In other approaches, protein antigens denatured with urea (5) or chemically modified by acetoacetylation (2) suppressed also specifically the IgE responses. Moreover, pollen allergens were rendered immunosuppressive by treating the allergens with either formaldehyde (7) or glutaraldehyde (8,9). The induction of immunological unresponsiveness in the IgE class of antibody employing the various protocols described above, resulted, in some instances, in the generation of thymus-derived suppressor cells (Ts). Tolerance due to receptor blockade or to clonal abortion of antibody producing cells (B) was postulated in some of the experimental systems.

The use of antigens linked to the nonimmunogenic carrier, the copolymer of D-glutamic acid, D-lysine (D-GL) has been recently reported by Liu et al (10,11) in which they demonstrated that the conjugates preferentially affected thymus derived helper cells (Th) without inducing

detectable Ts cell activity. However, the effect of protein antigens linked to isologous gamma globulins on the production of IgE antibodies in animals has not been reported. Previously, Lee and Sehon (reviewed in 6) clearly demonstrated that haptens linked to isologous gamma globulins suppressed the primary and secondary anti-hapten IgE responses.

The attempts in dampening the reaginic antibody response by the administration of soluble antigen has met with little success. Various investigators (6,11,12) demonstrated that the IgE response was only transiently suppressed by soluble antigen. In this laboratory preliminary results indicated that soluble dog albumin (DA) as well as conjugates of DA coupled to polyethylene glycol (DA-PEG) suppressed the anti-DA IgE response. Therefore, two systems were developed in the present study, in order to determine the effect of ovalbumin-murine isologous gamma globulin (OA-MYG) conjugates and soluble DA on the reaginic response.

Indeed, the results of this investigation demonstrated that treatment with conjugates of OA-MYG of normal mice or of mice presensitized with DNP-OA to a state of immediate hypersensitivity, suppressed the ability of these mice to produce anti-OA IgE antibodies and IgE antibodies to the hapten, 2,4 -dinitrophenyl (DNP), when the latter was presented to the immunological system with OA as a carrier. However, presentation of this hapten on a different carrier led to normal anti-DNP and anti-carrier IgE responses, suggesting that this type of tolerance was carrier-specific. Moreover, experimental results in this study indicated that this mode of immunosuppression was at least in part mediated by suppressor cells.

In the parallel study, soluble DA was found to induce a specific, long-lasting unresponsive state in normal mice or mice presensitized with

DA even upon two subsequent sensitizations with DA. This state was at least partially mediated by the dampening of Th cell function since mice primed with DNP-DA, after their treatment with soluble DA were unable to form anti-DNP IgE antibodies. Moreover, in an adoptive transfer system, T cells from animals treated with soluble DA were not capable of providing the necessary helper function to B cells from mice primed with DA in order to elicit anti-DA IgE response. In contrast, T cells from mice primed with DA provided helper function to B cells either from treated mice or mice primed with DA. It was also shown that antigen specific Ts cells were at least partially responsible for the unresponsive state. In addition, a factor extracted from the Ts cell abrogated the anti-DA IgE response in an adoptive transfer system.

LITERATURE REVIEW

The humoral responses to antigens are mainly categorized into two, depending upon the type of antigen used for elicitation of the response, namely thymus dependent or independent. Antigens which depended on thymus derived (T) cell interaction with bursa of Fabricius-derived cells (B) for antibody synthesis were categorized as T-dependent whereas, T-independent antigens induced an immune response without the apparent participation of T lymphocytes. The early studies of Humphrey et al (13) revealed that some antigens elicited immune responses to the capsular polysaccharide of type III pneumococci in the absence of T cells. However, Claman and co-workers were among the first to provide evidence for T-B cell interactions in the humoral responses of mice to the T-dependent antigen, sheep red blood cells (SRBC) (14-16). Thus, lethally irradiated recipients were injected intravenously with varying numbers of either spleen, thymus, bone marrow cells or a mixture of thymus and bone marrow cells, from normal or immune syngeneic donors, followed by antigenic challenge with SRBC. The recipients were bled at various times after cell transfer, for the determination of hemolytic activity in their sera. Hence, Claman and associates postulated from their results that the marrow population contained "effector cells" capable of producing antibody, but only in the presence of "auxiliary cells" present in the thymus population.

Support for this interpretation was forthcoming from Miller and Mitchell's laboratory (17). They reported that bone marrow cells of neonatally thymectomized mice were as effective as bone marrow cells of normal mice in restoring immunological responsiveness of heavily irradiated mice with intact thymuses. Furthermore, the immune response to SRBC was restored upon the administration of allogeneic thoracic duct or thymus

lymphocytes to neonatally thymectomized recipients. However, anti-H2 sera against host cell antigens, inhibited in vitro plaque forming cell (PFC) response to SRBC but anti-H2 antibodies directed against donor thoracic duct or thymus cells did not affect the response (18). In addition, they also established that precursors were indeed derived from the bone marrow population and moreover, that thymus cells recognized and reacted specifically with antigen, but performed an auxiliary role in the production of antibody by B cell precursors.

The pioneering work of Mishell and Dutton (19,20) and Marbrook (21) in which they elicited immune responses in vitro provided a useful tool in the study of specific cellular interactions to particulate antigens i.e. SRBC, and to soluble antigens, i.e. human serum albumin (HSA). Hence, the role of adherent and non-adherent cells in immune responses was studied (22,23). Subpopulations of splenic cells were obtained on the basis of their relative abilities to adhere to plastic or glass surfaces. These populations of cells developed a poor primary in vitro anti-SRBC response; however, remixing the two populations, the response was restored. In addition, the cell type in the adherent and nonadherent populations required for the in vitro response was identified as the macrophage and B,T lymphocytes respective. Furthermore, spleen cells from adult thymectomized, lethally irradiated mice reconstituted with syngeneic bone marrow cells were not capable of producing an in vitro response to SRBC. However, the in vitro response was restored upon reconstituting the mice with a thymus graft.

These initial studies which demonstrated the requirement for cellular interactions in the response to SRBC were followed by analogous findings in the development of humoral in vitro responses to soluble

protein antigens and perhaps more importantly to hapten-carrier conjugates (24-26). Landsteiner (27) introduced defined haptenic determinants onto immunogenic carriers and provided a valuable tool for the analysis of specific interactions between antigens and specific cells of the immune response. Briefly, immunization with a hapten-carrier conjugate elicited an anti-hapten response only when the hapten was coupled to an immunogenic carrier; nonimmunogenic carrier served only poorly or not at all, (28,30). Moreover, the induction of optimal hapten-specific secondary responses required the second challenge with the same hapten-carrier conjugate as that used for the primary immunization (31). The anti-hapten antibodies produced by such immunizations were highly specific for the haptenic determinant employed. Therefore, the assumption was made that the specificity of the antibody accurately expressed the specificity of the antigen-binding receptor molecules on the antibody forming cell precursor. Thus, the latter observations suggested the operation of an additional recognition mechanism for the carrier molecule. Indeed, cooperative interactions which were essential for the development of anti-hapten immune responses, were demonstrated between distinct lymphocytes, specific for carrier and haptenic determinants.

The first direct evidence for cooperative participation of two cells with distinct determinant specificities in the humoral response to hapten-carrier conjugates was initially obtained by Mitchison (32). Briefly, spleen cells from syngeneic donors, previously immunized either with a hapten-carrier conjugate or a second unrelated carrier injected together into irradiated recipients, produced an anti-hapten response when sensitized with the hapten heterologous carrier conjugate. In contrast, recipients of either cell population, which were immunized

with the heterologous hapten carrier conjugate, did not produce any anti-hapten response. Therefore, the cooperation of carrier-specific cells and anti-hapten antibody producing cells was essential for maximal anti-hapten antibody production.

Raff (33) demonstrated that the carrier-specific cooperating cells or "helper cells" were induced in the thymus, whereas anti-hapten antibody forming cells were not. Thus, Raff employed the identical protocol as described by Mitchison (32) and demonstrated that the carrier specific helper cells were T cells since the cells were sensitive to anti-theta and complement whereas the anti-hapten antibody producing cells were not sensitive to the treatment. Hence, by extension, B lymphocytes were considered to be the hapten antibody producing cells.

Conclusive evidence which showed that the B lymphocytes lineage were the progenitors of antibody secreting cells, came from studies using anti-sera specific for the mouse bone marrow lymphocyte marker (MBLA) in the mouse. Niederhuber et al (34) provided evidence that MBLA determinants existed on precursors of antibody forming cells, by employing an adoptive transfer studies of cooperative immune response to hapten-carrier conjugates. It was shown that hapten-specific B cells were susceptible to the cytotoxic effects of anti-MBLA antibodies but not anti-theta antibodies.

The same cooperation phenomenon between carrier-specific T cells and hapten-specific B cells was shown by a somewhat different approach and involving supplemental immunization of the intact animal with free carrier. Thus, rabbits immunized with p-azobenzene sulfonic acid (sulfanil) derivative of bovine serum albumin (BSA) made significant secondary anti-sulfanil antibody responses to sulfanil-human gamma globulin (HGG), when the rabbits had received a supplemental intervening

immunization with the free, unconjugated carrier, HGG (35). These results were subsequently confirmed by Katz (36,37) in rabbits and in guinea pigs.

A subset of T cells were involved also in the negative regulation of the immune response. These cells were first described by Gershon (38-40) and have been named Ts cells. Two broad categories of Ts cells are defined on the basis of functional effects of the cells, i.e. specific and nonspecific cells. The antigen specific Ts cells were defined as such, since, their induction and biological effects were restricted to response related to specific antigenic determinants. On the other hand, nonspecific Ts cells are not restricted in their effects or function, irrespective of whether or not the Ts cells were specifically activated by an antigen (41).

Gershon and Kondo (42,43) observed in mice the activity of Ts cells in the development of immunological unresponsiveness to SRBC. The effects of Ts cells tended to be essentially the opposite of Th cell. Among the first studies which demonstrated a dualistic influence of carrier-specific T cells in response to hapten-carrier conjugates were those performed by Katz et al (44) and Tada (45). Thus, guinea pigs were primed with soluble DNP-OA on day 0 and one week later, the various groups were immunized with BGG or keyhole limpet hemocyanin (KLH) or a mixture of BGG and KLH emulsified in Freund's complete adjuvant (FCA) (44). Three weeks later, the guinea pigs were injected intraperitoneally (i.p.) with 10 mg of soluble BGG or KLH followed one day later by a secondary challenge with DNP-BGG. The supplemental immunization with BGG in FCA induced a population of BGG-specific T cells capable of regulating the response to DNP-BGG. The net effect resulted in a very strong secondary

anti-DNP response. However, administration of soluble BGG 1 day prior to the secondary immunization abrogated the anti-DNP response of the recipients. The unresponsive state was antigen specific since administration of unconjugated KLH to guinea pigs preimmunized with BGG in FCA failed to suppress the response. However, guinea pigs treated with KLH in FCA and resensitized with DNP-BGG showed clearly a depressed anti-DNP response demonstrating a nonspecific dampening of the anti-DNP response by KLH.

Moreover, a specific unresponsive state was induced in rats administered the carrier prior to sensitization with the homologous hapten-carrier conjugate, whereas rats challenged with a heterologous hapten-carrier conjugate produced a primary anti-hapten antibody response (45). The unresponsive state induced in these animals was mediated by antigen specific Ts cells. Hence, rats treated with KLH and challenged with DNP-BGG produced an anti-DNP antibody response.

Moreover, Tada and associates extracted a soluble suppressive factor from the Ts cell. The factor did not contain any immunoglobulin determinants and had a molecular weight between 35,000 and 60,000 daltons (46,47). These early results prompted Tada and his collaborators to study the factor more closely. They opted for an in vivo cell transfer system employing inbred mice and extracted a soluble factor from Ts cell induced in mice primed earlier with KLH (47-49). This Ts cell factor had very similar effects and properties as the Ts cell factor obtained in rats.

The genetic nature of the Ts cell factor was studied by using various inbred strains of mice differing in H-2 haplotypes and the genetic requirements for the interaction of suppressor and acceptor molecules were elucidated. Tada and co-workers first demonstrated by employing allo-

antisera to the various subregions, that the unique Ts cell factor was a product of a restricted subregion which was later defined as I-J subregion of the I region of the H-2 complex (50,51).

Moreover, they clearly demonstrated that: 1) the Ts cell factor was effectively absorbed with alloantisera directed to I region of the H-2 complex; 2) alloantisera against histocompatibility antigens coded for by K or D region genes did not absorb the Ts cell factor; 3) the absorbing capacity of alloantisera did not correlate with the anti-Ia specificities which had been determined by cytotoxic activity for B cells; 4) antisera containing specificity for I-J subregion gene products absorbed the suppressive activity, whereas those lacking I-J specificity did not; 5) the Ts cell factor obtained from one strain of mice effectively suppressed the responses of other strains sharing the same I-J subregion and 6) the acceptor site for the Ts cell factor was also postulated to be coded for by genes in the I-J subregion. It was suggested that the I-J subregion genes coded for complementary cell surface molecules on different subsets of lymphoid cells through which suppressive interaction was effectively achieved (52,53).

One of the more striking systems involved in the activity of Ts cells has been that of the genetically controlled response to the synthetic terpolymer, GAT. Antibody responses to GAT are controlled by an autosomal dominant Ir gene mapping in the I region of the H-2 complex. Gershon et al (54) initially postulated that the cellular basis for unresponsiveness in nonresponder strains of mice to GAT was related to the existence of Ts cells inhibiting the capacity of such animals to produce antibodies to GAT. The hypothesis was based upon the ability of GAT to stimulate DNA synthesis in vitro, following primary immunization