# SUPPRESSION OF IgE ANTIBODY RESPONSES BY GRAFT-VERSUS-HOST REACTIONS

by

PARVIZ PAKZAD

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for

the Degree of Doctor of Philosophy

DEPARTMENT OF IMMUNOLOGY

THE UNIVERSITY OF MANITOBA

WINNIPEG, MANITOBA

JANUARY, 1980

# SUPPRESSION OF IgE ANTIBODY RESPONSES BY GRAFT-VERSUS-HOST REACTIONS

BY

PARVIZ PAKZAD

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

To my parents and my

wife Leila who have

offered their encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

TABLE OF CONTENTS - Continued

TABLE OF CONTENTS - Continued

## ABSTRACT

### Suppression of IgE antibody responses by graft-versus-host reactions

The failure of $F_1$ hybrid mice undergoing graft-versus-host reactions (GvHR), caused by the transfer of parental spleen cells, to respond to an antigen was used as a model in this dissertation to study the regulation of IgE antibody responses. The formation of IgE as well as hemagglutination (HA) antibody responses was suppressed in $B_6D_2F_1$ ($H\text{-}2^{b/d}$) mice by an intraperitoneal (i.p.) inoculation of parental C57BL/6 ($H\text{-}2^{b}$) spleen cells. The suppression was effective when the donor cells were transferred 3 days before or after or even 1-2 hours (hr) before administration of an i.p. sensitizing dose of $DNP_3$-OA. It was demonstrated that the splenic T cells of the C57BL/6 parent were responsible for the immunosuppression in $B_6D_2F_1$ recipients and by using several strains of inbred mice with different haplotypes as donors, it was suggested that $H\text{-}2^{b}$ haplotype played an important role in GvH-induced immunosuppression.

The measurement of spleen index at different times following the induction of GvHR by different parental spleen cells revealed that C57BL/6 spleen cells induced stronger GvHR than DBA/2 parental spleen cells and maximum spleen

enlargement of $B_6D_2F_1$ mice occurred at about 9 days following cell transfer. Furthermore, transfer of spleen cells of $F_1$ mice, which had received parental C57BL/6 spleen cells at different times prior to sacrifice, in combination with $DNP_3$-OA primed $F_1$ spleen cells into irradiated $F_1$ recipients suggested that there was a close correlation between the degree of immunosuppression and spleen enlargement. Further studies demonstrated that the suppressor cells which were generated during the course of a GvHR were T cells in nature and they originated from the grafted donor T cells. Some characteristics of the parental cells which induced immunosuppression in the $F_1$ hybrid mice as well as the properties of the suppressor T cells which were generated by GvHR, and the role of their suppressor factors were also described.

## ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to the following persons to whom I owe a debt of gratitude:

to my advisor Dr. W. Y. Lee for his patient guidance and encouragement throughout this investigation and also the preparation of this thesis;

to Dr. E. Sabbadini for his valuable criticism and direction during the course of this study as well as the preparation of this manuscript;

to Dr. A. H. Sehon who made possible my studies in this department;

to the University of Jundishapur for the award of the Scholarship and the University of Manitoba for the award of the Manitoba Graduate Fellowship during my study in this department;

to Miss Dawn Hubbard and Mr. Stephan Krueger for correcting and proof-reading the script;

and to my wife Leila for her constant understanding and encouragement during the difficult periods of research.

## LIST OF FIGURES

LIST OF FIGURES - Continued

LIST OF TABLES - Continued

## ABBREVIATIONS

| | | |
|---|---|---|
| $Al(OH)_3$ | : | aluminum hydroxide |
| ALS | : | anti-lymphocyte serum |
| ASF | : | allogeneic suppressor factor |
| ASC | : | extract of *Ascaris suum* |
| ATS | : | anti-thymocyte serum |
| B | : | bursa of Fabricius equivalent cell |
| $B_\varepsilon$ | : | B-cell producing IgE antibodies |
| $B_\gamma$ | : | B-cell producing IgG antibodies |
| BGG | : | bovine gamma globulin |
| B.M. | : | bone marrow |
| BPO | : | benzylpenicilloyl group |
| BSA | : | bovine serum albumin |
| C' | : | complement |
| cm | : | centimeter |
| CML | : | cell-mediated lympholysis |
| Con A | : | concanavalin A |
| DA | : | dog serum albumin |
| D-GL | : | synthetic random copolymer of D-glutamic acid and D-lysine |
| DNBS | : | sodium salt of 2,4 dinitrobenzene sulfonic acid |
| DNP | : | 2,4-dinitrophenyl group |
| DNP-Tbc | : | dinitrophyenylated mycobacterium |
| DTH | : | delayed type hypersensitivity |

ABBREVIATIONS - Continued

| | | |
|---|---|---|
| ECF-A | : | eosinophil chemotactic factor of anaphylaxis |
| EFA | : | enhancing factor of allergy |
| FCA | : | Freund's complete adjuvant |
| FCS | : | fetal calf serum |
| Fig. | : | figure |
| FUdR | : | 5-fluorodeoxyuridine |
| g | : | gram |
| x g | : | times the factor of gravity |
| GAT | : | synthetic random copolymer of glutamic acid, alanine and tyrosine |
| GT | : | synthetic copolymer of glutamic acid and tyrosine |
| GvHR | : | graft-versus-host reactions |
| H-2 | : | major histocompatibility complex of the mouse |
| HA | : | hemagglutination |
| HCl | : | hydrochloric acid |
| HEPES | : | N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid |
| PBM | : | peripheral blood mononuclear cells |
| hr | : | hour |
| HRBC | : | horse red blood cells |
| HSA | : | human serum albumin |
| Ig | : | immunoglobulins |
| IgE-TsF | : | IgE class specific suppressor T cell factor |
| i.d. | : | intradermal |
| i.m. | : | intramascular |
| i.p. | : | intraperitoneal |

ABBREVIATIONS - Continued

| | | |
|---|---|---|
| i.v. | : | intravenous |
| $^{125}$IUdR | : | 5-($^{125}$I) Iodo-2'-deoxyuridine |
| KLH | : | keyhole limpet haemocyanin |
| $KH_2PO_4$ | : | potassium phosphate monobasic |
| M | : | molar |
| MEM | : | minimum essential medium |
| mg | : | milligram |
| MHC | : | major histocompatibility complex |
| µg | : | microgram |
| µl | : | microliter |
| ml | : | milliliter |
| mm | : | millimeter |
| mM | : | millimolar |
| MLR | : | mixed leukocyte reaction |
| MγG | : | mouse gamma globulin |
| MΦ | : | macrophage |
| MRBC | : | mouse red blood cells |
| NaCl | : | sodium chloride |
| $Na_2CO_3$ | : | sodium carbonate |
| $NaN_3$ | : | sodium azide |
| NaOH | : | sodium hydroxide |
| $Na_2HPO_4$ | : | sodium phosphate dibasic |
| $(NH_4)_2SO_4$ | : | ammonium sulfate |
| nm | : | nanometer |
| NMS | : | normal mouse serum |

ABBREVIATIONS - Continued

| | | |
|---|---|---|
| NRS | : | normal rabbit serum |
| NTx | : | neonatally thymectomized |
| OA | : | ovalbumin |
| PAF | : | platelet-activating factor |
| PBS | : | phosphate-buffered saline |
| PCA | : | passive cutaneous anaphylaxis |
| PEG | : | polyethylene glycol |
| PFC | : | plague-forming cells |
| PHA | : | phytohemagglutinin |
| PLL | : | synthetic random polymer, poly-L-lysine |
| POL | : | polymerized flagellin |
| R | : | rads of radiation |
| Rag | : | extract of ragweed pollen |
| rpm | : | revolutions per minute |
| SIII | : | penumococcus polysaccharide, type III |
| Salm | : | salmonella bacilli |
| SAS | : | saturated ammonium sulfate |
| SFA | : | serum molecule suppressive factor of allergy |
| S.I. | : | spleen index |
| SRBC | : | sheep red blood cells |
| SRS-A | : | slow reacting substance of anaphylaxis |
| T | : | thymus derived cells |
| (T-G)-A--L | : | synthetic branched polymer of tyrosine, glutamic acid, alanine, and lysine |
| UD | : | urea-denatured |
| vs | : | versus |

# INTRODUCTION

Injection of parental immunocompetent lymphoid cells into $F_1$ hybrid mice results in a graft-versus-host reaction (GvHR). The host is tolerant to parental alloantigens and is therefore, considered to be unreactive towards the graft (Simonsen 1962). The GvHR represents a unique complex model for studying the dynamics of cellular events that occur following the specific stimulation of antigen reacting lymphocytes. This stimulation may lead to a strong suppression of the immune response of the host by means of one of several regulatory mechanisms such as:

1. generation of suppressor cells (Shand 1975; 1976; 1977; Pickel and Hoffmann 1977a; b),
2. generation of suppressor factors (McMaster and Levy 1975; Yonkoskey et al. 1976a; b) or,
3. depletion of either an essential cell population (Lapp et al. 1974) or factors (Parthenais et al. 1974).

The GvH-induced immunosuppression has been shown to suppress both in vivo (Blaese et al. 1964; Lapp et al. 1974; Moller 1971; Shand 1975) and in vitro (Sjöberg 1971; Sjöberg 1972; Elie et al. 1974; Parthenais et al. 1974; Shand 1976) humoral immune responses, as well as cell-mediated immunity (Howard and Woodruff 1961; Lapp and Moller 1969; Treiber and Lapp 1973) and tumor immunity (Solnik et al. 1973).

It has also been demonstrated that the early stages of allogeneic interaction stimulate the humoral immune response to thymus dependent antigens (Katz et al. 1971b). However, it was shown that a GvHR induced by the injection of murine parental lymphoid cells into a $F_1$ recipient can both potentiate and inhibit the humoral immune responses to an antigen (Byfield et al. 1973; Pickel and Hoffmann 1977a).

In the past 2 years, efforts in clinical bone marrow transplantation have increased significantly, as evident by the fact that several new centers, both in U.S. and in Europe, have initiated a bone marrow transplantation program and others are preparing to do so (Van Bekkum et al. 1979). However, GvHR remains to be a severe limitation to the success of this procedure and to the more general application of bone marrow grafting. The precise immunoregulatory events which are operative during the course of GvHR or GvH disease in patients with either leukemia, aplastic anaemia or an immunodeficiency, and in patients grafted with histoincompatible lymphoid cells, are still unknown.

GvHR has also been demonstrated during the course of pregnancy when there is a sufficient histoincompatibility between mother and fetus, and that under certain circumstances the maternal lymphocytes may pass through the placental barrier and stimulate a demonstrable GvH disease (Beer and Billingham 1978; Griffin et al. 1978).

An increasing number of immunological responses have been shown to be under genetic control. In general, these responses have been linked to the major histocompatibility complex (MHC), and it appears that the gene products of the MHC are controlling the host immune responses to a variety of antigens (Katz 1977) as well as the recognition of self and nonself antigens (Benacerraf and Katz 1975).

The production of IgE antibody responses have been shown to be dependent on the histocompatible co-operation between thymus derived (T) and bursa equivalent (B) cells (Kishimoto and Ishizaka 1972a; Hamaoka et al. 1973). However, it is generally agreed that the induction of an IgE antibody response is dependent on several factors such as doses of antigen, types of adjuvants, routes of injection etc. The collective experimental evidence so far suggests that there are at least three ways of dampening the formation of IgE antibody responses: (a) the generation of suppressor cells e.g. by high doses of antigens (Takatsu and Ishizaka 1976; Schwenk et al. 1979) or by modified antigens (Takatsu and Ishizaka 1975; Lee and Sehon 1978a; b; Usui and Matuhasi 1979b); (b) the receptor blockade of B cells by either hapten-coupled-nonimmunogenic carrier (Lee and Sehon 1975b), hapten coupled-immunogenic carrier (Danneman and Michael 1977) or hapten-coupled T independent antigens (Shinohara and Tada 1974; Tada 1975); and (c) the generation of suppressor

factors by i.p. inoculation of either Freund's complete adjuvant (FCA) (Tung et al. 1978) or allogeneic lymphoid cells (Katz 1979).

The failure of histoincompatible cells to respond to an antigen during the course of GvHR was used as a model in this dissertation to study the regulation of IgE antibody responses. The primary objective of this study was to investigate the mechanism of suppression of IgE antibody responses in GvHR. In this connection, the contribution of various types of parental lymphoid cells and the cells of other genotypes in the suppression of IgE antibody responses in $F_1$ mice was studied. Some characteristics of the parental cells which induced immunosuppression in the $F_1$ hybrids as well as the characteristics of the suppressor T cells and the suppressor factors which were generated in GvHR were also described. The relationship between spleen enlargement during the course of GvHR and suppression of IgE antibody responses was also studied in an adoptive transfer system. The suppressor T cell was suggested to originate from the parental cells in GvH-induced immunosuppression.

# REVIEW OF LITERATURE

## IgE-MEDIATED IMMEDIATE TYPE HYPERSENSITIVITY

Mota (1961, 1964) first described the formation of reaginic antibodies to protein antigens in the presence of Bordetella pertussis, an adjuvant, in the rat. Later, Ishizaka and co-workers (1966) were the first to find a distinct reaginic immunoglobulin class which they termed immunoglobulin E (IgE, E stands for erythema) in the serum of hay fever patients.

It has been suggested that IgE-mediated immediate type hypersensitivity occurs as a result of non-covalent binding of IgE molecules, via Fc receptors, to the tissue mast cells or blood basophils (Ishizaka et al. 1970b; Ishizaka and Ishizaka 1974). Bridging of the Fab regions of these IgE molecules by either specific di- or polyvalent allergens or divalent anti-IgE antibodies results in release of vasoactive mediators of anaphylaxis such as histamine, slow reacting substance of anaphylaxis (SRS-A), eosinophil chemotactic factor of anaphylaxis (ECF-A), and platelet-activating factor (PAF) from the mast cells and basophils (Ishizaka et al. 1972; Ishizaka and Ishizaka 1969). The IgE molecules on the surface of the basophils can be detected by autoradiography using $^{125}$I-labeled anti-IgE antibody

(Ishizaka et al. 1970b), or by electron microscopy (Sullivan et al. 1971). It has also been shown that monovalent hapten induces neither anaphylaxis nor reaginic hypersensitivity reactions (Levine and Rodmond 1968). The direct bridging of the cell receptor molecules by divalent anti-receptor antibody causes histamine release in a non-cytotoxic way. However, binding of the monovalent antibody fragments with the receptor does not result in histamine release (Ishizaka et al. 1978b).

## FACTORS INFLUENCING THE INDUCTION OF IgE ANTIBODY RESPONSES

At present time IgE type reagins have been identified in man (Ishizaka and Ishizaka 1967); monkey (Ishizaka et al. 1969); rabbit (Strannegard and Chan 1969; Ishizaka et al. 1970a); rat (Mota 1964; Stechschulte et al. 1970); mouse (Mota 1967; Prouvost-Danon et al. 1972); guinea pig (Levine et al. 1971; Margni and Hajos 1973); dog (Patterson and Sparks 1962; Tse et al. 1978); sheep (Hogarth-Scott 1969); pig (Barratt 1972) and cow (Hammer et al. 1971), but the presence of their counterpart in avian species has not been confirmed. Although the IgE antibodies in these species have common characteristic properties, the conditions for the induction the IgE antibody are different among various species and depends on many factors. These factors are as follows:

1) Nature of adjuvant: It has been known that the nature of adjuvant used to induce IgE antibody responses is extremely important. Among many adjuvants, $Al(OH)_3$ (Revoltella and Ovary 1969; Levine and Vaz 1970; Kishimoto and Ishizaka 1973b; Lee and Sehon 1975a) and Bordetella pertussis (Mota and Peixoto 1966; Okumuro and Tada 1971) have been found to be most useful in the induction of the IgE antibody responses, whereas the FCA fails to elicit or elicits very poorly IgE antibody responses (Kishimoto and Ishizaka 1973b; c; Katz 1978). Gollapudi and Kind (1975a; b; 1977a) demonstrated that injection of OA in combination with Concanavalin A (Con A) as adjuvant induced reaginic antibody responses in a number of strains of mice.

2) Nature of antigen: Some antigens have been shown to be superb in the induction of IgE antibody responses. Among those which have been tested are Ascaris extract (ASC) and ovalbumin (OA), whereas keyhole limpet haemocyanin (KLH), FCA, bovine serum albumin (BSA) and bovine gamma globulin (BGG) have not been found to be good antigens for IgE antibody responses (Strejan et al. 1973; and Lee and Sehon 1975a; Katz 1978).

3) Dose of antigen: It was found that low doses of antigen induce optimum IgE antibody responses, whereas higher doses of antigen cause suppression of IgE antibody responses (Takatsu and Ishizaka 1976a; Schwenk et al. 1979).

4) Species of the animals: Not all experimental animals are good IgE antibody producers. For example, it is difficult to produce IgE antibody in guinea pigs, whereas most strains of mice readily produce IgE antibody (Levine et al. 1971; Margni and Hajos 1973).

5) Genetic make up of the animal: The genetic phenotype of inbred animals also plays an important role in IgE antibody responses. In the case of mice, the inbred strains have been classified as either low IgE responder phenotypes like SJL mice, or high IgE responder phenotypes like $B_6D_2F_1$ mice (Levine and Vaz 1970).

6) Route of administration of antigen: It has been demonstrated that the i.p. inoculation of an antigen in combination with an appropriate adjuvant is most favorable for the production of IgE antibody responses (Mota 1967; Ishizaka and Okudaria 1973; Lee and Sehon 1975a). The IgE antibody responses by lymphocytes from Peyer's patches in mice have also been detected by the heterologous adaptive cutaneous anaphylaxis (HACA) test (Kind and Maceda-Sobrinho 1973) upon tracheal and subcutaneous administration of antigen in an appropriate adjuvant (Gerbrandy and Bienenstock 1976). However, the oral administration of an antigen has been shown to induce suppression of IgE antibody responses to the corresponding antigen (David 1977; Vaz et al. 1977; Ngan and Kind 1978).

## REGULATION OF IgE ANTIBODY RESPONSE

It has been well documented that collaboration between two types of lymphocytes, namely, bone marrow derived precursors of antibody forming cells (B-cells) and thymus derived lymphocytes (T-cells) is essential for the induction of antibody responses to most protein antigens (Claman and Chaperon 1969; Miller and Mitchel 1969; Katz and Benacerraf 1972). The fundamental processes of cells interacting in IgE antibody responses are the same as those observed in IgM and IgG antibody responses. However, certain unique features exist with respect to the influence of T cells on IgE B cells ($B_\varepsilon$) which will be discussed later.

As in IgG antibody production, helper T cells usually possess carrier specificity in anti-hapten IgE antibody responses. Using such hapten-carrier systems, it has been demonstrated that T-cells primed with carrier molecules, "help" the hapten-specific precursor $B_\varepsilon$ cells to differentiate into antibody producing cells (Hamaoka et al. 1973; Kishimoto and Ishizaka 1972a).

## ROLE OF T CELLS AND B CELLS IN PRODUCTION OF REAGINIC ANTIBODY RESPONSES

Although congenitally athymic (nu/nu) mice can produce IgM antibodies against a variety of antigens, such mice have

been found to be unable to produce IgE antibody against the OA antigen (Michael and Bernstein 1973). However, the administration of normal thymus cells or the grafting of the thymus into these animals initiated the formation of IgE antibody responses. Similarly, neonatally thymectomized rats (thymectomized within 24 hours of birth) could not produce IgE antibody response upon subsequent immunization with a hapten-carrier conjugate, but transfer of normal as well as carrier primed thymocytes into such neonatally thymectomized rats could restore their ability to produce anti-hapten IgE antibody responses (Okumura and Tada 1971).

It has been demonstrated that both T and B lymphocytes are necessary for IgE antibody formation (Tada 1975). This has been shown in lethally irradiated rats which have been reconstituted with B.M. or thymus cells or both cell types. Only animals given B.M. cells, in combination of thymus cells produce IgE antibody response to hapten-carrier conjugates.

Although B lymphocytes are the cells that eventually differentiate to become antibody producing cells, it is actually the T cells that control the magnitude and duration of the antibody response to a given antigen. It has been shown that the pre-immunization of animals with a carrier, along with certain adjuvants, enhances the anti-hapten IgE antibody responses upon primary immunization of the hapten coupled to the same carrier (Tada et al. 1972; Kishimoto