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SUPPRESSION OF THE IgE ANTIBODY RESPONSE WITH MODIFIED AND SOLUBLE ANTIGEN

by

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TO THE LORD GOD

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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 (MγG) 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-MγG, 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

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.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

$Al(OH)_3$: aluminum hydroxide

ALS : anti-mouse lymphocyte serum

anti- : antibody

α-A : α-amylase

Asc : *Ascaris suum* extract

B : bursa of Fabricius equivalent cell

B_ε : bursa of Fabricius equivalent cell producing IgE

BBS : borate buffered saline

BGG : bovine gamma globulin

BPO : benzylpenicilloyl group

BSA : bovine serum albumin

$B_6D_2F_1$: $(C_{57}Bl/6 \times DB\ A/2)F_1$ 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

Ig : immunoglobulin

IgG : immunoglobulin of the G class

IgE : immunoglobulin of the E class

^{125}I : radioactive istotope 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. : intraveneously

KLH : keyhole limpet hemocyanin

MEM : minimal essential medium

2ME : 2- mercaptoethanol

ml : milliliter

mg : milligram

MγG : 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-MγG : ovalbumin conjugated to MγG

OA-RγG : ovalbumin conjugated to RγG

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

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

µg : microgram

µl : 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-MγG) conjugates and soluble DA on the reaginic response.

Indeed, the results of this investigation demonstrated that treatment with conjugates of OA-MγG 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 pneumoccoci 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 intraveneously 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 reconstitued 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