LECTURE: 30

Title   REGULATION OF THE IMMUNE RESPONSE

LEARNING OBJECTIVES:

The student should be able to:

- **Enumerate the different type of cells involved in the regulation of the immune response.**

- **Explain the regulation of the immune response by antigen:**

T-dependent & T-independent antigens, Dose of the antigen, route of administration, type of the antigen: Pure lipids, pure proteins, glycoproteins, and carbohydrates.

- **Explain the regulation of the response by cell mediated immunity**

- Antigen presenting cells: (antigen recognition on the APCs),
- Activation of Th CD4$^+$ T cells (memory & effector cells).

- **Explain the regulation of the immune response by antibody:**

     - Mechanism involved in antibody production, Cooperation between involved cells (B & T-helper), Passive administration of IgM, and IgG with antigens, Immune complexes (enhance or suppress immune responses).

- **Explain the regulation of the immune response by lymphocytes:**

- **CD4$^+$ Th2 cell is a normal physiological process [Activation of Tc, and Ts, and Activation of B cells (by T-dependent antigens].**

- **Explain the regulation of the immune response by idiotypic modulation.**
- **Explain generally the regulation by neuroendocrine modulation of immune responses.**
- **Explain generally know the regulation by genetic control of immune responses.**

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE
IMMUNOLOGY. 6th edition. Chapter 11. pg. 173-189.

2. HANDOUT.

REGULATION OF THE IMMUNE RESPONSE

The effective immune response is an outcome of the interplay between antigen and a network of immunologically competent cells. The immune response is controlled by many mechanisms which restore the immune system to a resting state when responsiveness to a given antigen is no longer required.

Effective immune responses are usually resulted from the interactions between pathogens and a network of immunologic elements. All these responses, which are created as a result of a challenge with infective microbe, are matter to several different control mechanisms. These mechanisms return the immune system to the resting state after removal of the microbe is occurred and the immunologic actions of these elements to the removed microbe are no longer needed.

The immune response, like all biological systems, is subject to a variety of control mechanisms. These mechanisms restore the immune system to a resting state when responsiveness to a given antigen is no longer required. An effective immune response is an outcome of the interplay between antigen and a network of immunological competent cells. The nature immune of immune response, both qualitatively and quantitatively, is determined by many factors, including the form and route of administration of the antigen, the antigen-presenting cell (APC), the genetic background of the individual and any history of previous exposure to the antigen in question or to a cross-reacting antigen. Specific antibodies may also modulate the immune response to an antigen. Some of these factors are discussed in detail elsewhere and are dealt with only briefly here.

GENERAL INFORMATION

- The immune response is subject to a variety of control mechanisms which serve to restore the immune system to a resting state when the response to a given antigen is no longer required.

- Many factors govern the outcome of any immune response. These include the antigen itself, its dose and route of administration, and the genetic background of the individual responding to antigenic challenge.

- Immunoglobulins can influence the immune response positively as anti-idiotype or through immune complex formation. They may also negatively influence immune responses by reducing antigenic challenge or by feedback inhibition of B cells.

- The antigen-presenting cell may affect the immune response through its ability to provide co-stimulation to T cells. Different types of antigen-presenting cell promote different modes of immune response.

- T cells regulate the immune response. Cytokine production by T cells influences the type of immune response elicited by antigen. $CD4^+$ T cells can deviate immune responses to TH1 (T-1 helper) or TH2 type responses. Regulatory T cells may belong to the CD4 or CD8 subpopulations. They can inhibit responses by production of suppressive cytokines such as interleukin 10 (IL-10) and transforming growth factor-β (TGFβ).

- Selective migration of lymphocyte subsets to different sites can modulate the local type of immune response, since TH1 cells and TH2 cells respond to different sets of chemokines.

- Genetic factors which influence the immune system include both MHC-linked and non-MHC linked genes. They affect the level of immune response, susceptibility to infection and autoimmune disease. Defects in many of these genes lead to immunodeficiency or abnormal immune responses.

- The neuroendocrine system influences immune responses. Corticosteroids in particular down-regulate TH1 responses and macrophage activation.

The nature of the immune response both quantitatively and qualitatively is regulated by many factors, these include:

A. Regulation by Antigens

Antigens include those which induce immune responses, and those which cause the state of tolerance.

T cells and B cells are triggered by antigen after effective engagement of their antigen-specific receptors together with appropriate co-stimulation. In the case of the T cell, this engagement is not with antigen itself, but with processed antigenic peptide bound to MHC class I or class II molecules on APCs. The nature of an antigen its dose and the route of administration have all been shown to have a profound influence on the outcome of an immune response. An effective immune response removes antigen from the system. Repeated antigen exposure is required to maintain T- and B-cell proliferation of during an effective immune response there is often a dramatic expansion of specifically reactive effector cells. At the end of an immune response reduced antigen exposure results in a reduced expression of IL-2 and its receptor leading to apoptosis of the antigen-specific T cells. The majority of antigen-specific cells therefore die at the end of an immune response and a minor population of long-lived antigen-specific T and B cells survives to give rise to the memory population of antigen-specific cells.

The nature of the antigen influences the type of immune response that occurs

Different antigens elicit different kinds of immune response. Polysaccharide capsule antigens of bacteria generally induce IgM responses, whereas proteins can induce both cell-mediated and humoral immune responses. Intracellular organisms such as some bacteria, parasites or viruses induce a cell-mediated immune response, whereas soluble protein antigens induce a humoral response. A cell-mediated immune response is also induced by agents such as silica.

However, some antigens (e.g. those of intracellular microorganisms) may not be cleared so effectively, leading to a sustained immune response that has pathological consequences.

Large doses of antigen can induce tolerance

Very large doses of antigen often result in specific T- and sometimes B-cell tolerance. It has been shown that administration of antigen to neonatal mice often results in tolerance to this antigen. It has been speculated that this may be the result of the immaturity of the immune system. However, recent studies have shown that neonatal mice can develop efficient immune responses (**Figure-1**) and that non-responsiveness may in some cases be attributable, not to be the immaturity of T cells, but to immune deviation whereby a non-protective type II cytokine response dominated a protective type I response. T-independent polysaccharide antigens have been shown to generate tolerance in B cells after administration in high doses.

Effect of antigen does on the outcome of the immune response to murine leukaemia virus

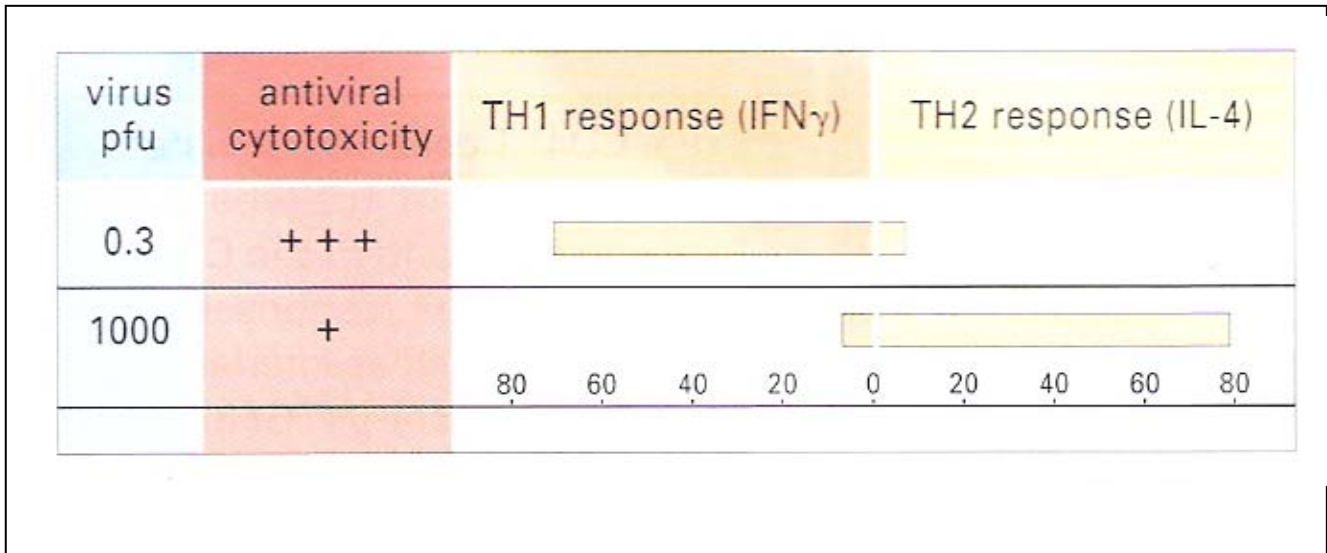| virus pfu	antiviral cytotoxicity	TH1 response (IFNγ)	TH2 response (IL-4)
0.3	+ + +		
1000	+		

Figure-1 Newborn mice were infected with either 0.3 or 1000 plaque-forming units (pfu) of virus and the CTL response against virally infected targets was assessed together with the production of IFNγ (TH1 cytokine) or IL-4 (TH2 cytokine) in response to viral challenge. Mice infected with a low dose of virus make a TH1-type response and are protected. The results are presented as arbitrary units.

The route of administration of an antigen can determine whether or not an immune response occurs

The route of administration of antigen has been shown to influence the immune response. Antigen administered subcutaneously or intradermally evokes an immune response, whereas those given intravenously, orally or as an aerosol may cause tolerance or an immune deviation from one type of CD4$^+$ T cell response to another. For example, rodents that have been fed ovalbumin or myelin basic protein (MBP) do not respond effectively to a subsequent challenge with the corresponding antigen. Moreover, in the case of MBP, the animals are protected from the development of the autoimmune disease, experimental allergic encephalomyelitis (EAE). This phenomenon may have some therapeutic value in allergy; recent studies have shown that oral administration of a T cell epitope of the Der p1 allergen of house dust mite (*Dermatophagoides pteronyssimus*) could tolerize to the whole antigen. The potential mechanisms of such tolerance induction include anergy, immune deviation and the generation of regulatory T cells that act through the production of cytokines such as TGFβ.

Similar observation has been made when antigen is given as an aerosol. Studies in mice have shown that aerosol administration of an encephalitogenic peptide inhibits the development of EAE that would normally be induced by a conventional (subcutaneous) administration of the peptide (**Figure-2**). This also may have therapeutic implications, as the inhibition of the response is not limited to the antigen administered as an aerosol, but also includes other antigens capable of inducing EAE such as protedipid protein.

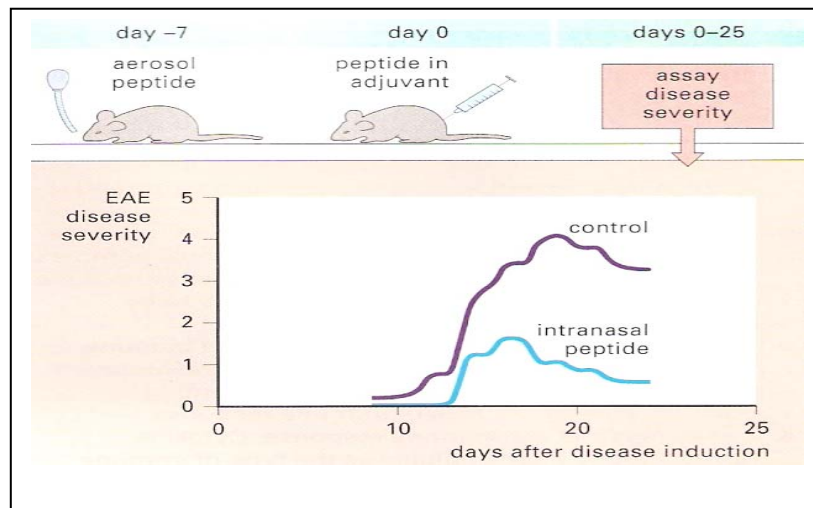# Aerosol administration of antigen modifies the immune response

Figure-2 Mice were treated with a single aerosol dose of either 100 µg peptide (residues 1-11 of myelin basic protein), or just the carrier. Seven days later the same peptide, this time in adjuvant, was administered subcutaneously. The subsequent development of EAE was significantly modified in pretreated animals.

A clear example of how different routes of administration affect the outcome of the immune response is provided by studies of infection with lymphocytic choriomeningitis virus (LCMV). Mice primed subcutaneously with peptide in incomplete Freund's adjuvant develop immunity to LCMV. However, if the same peptide is repeatedly injected intraperitoneally, the animal becomes tolerized and cannot clear the virus (**Figure-3**).

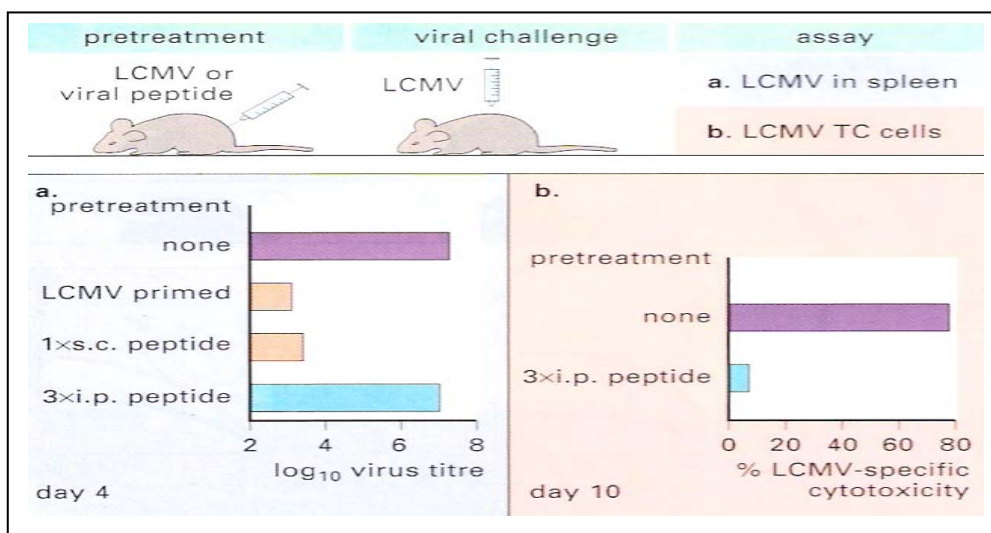## Peptide-induced inactivation of LCMV-specific T cells

Figure-3 Mice were either primed with LCMV or injected with 100 µg LCMV peptide. The peptide was given either subcutaneously (s.c.) or three times intraperitoneally (i.p.) with incomplete Freud's adjuvant. The animals were later infected with LCMV (day 0). The titre of virus in the spleen was measured on day 4. Animals that had been pretreated with subcutaneous peptide or with LCMV developed neutralizing antibody and protective immunity against the virus; animals pretreated with peptide i.p. did not develop immunity. Cytotoxic T cell activity was assessed in the mice on day 10. Mice that had receive no pretreatment demonstrated Tc cells specific for the LCMV peptide. Mice pretreated with peptide i.p. failed to show such activity.

THE ANTIGEN-PRESENTING CELL

The nature of the APC initially presenting the antigen may determine whether responsiveness or tolerance ensues. Effective activation of T cells requires the expression of co-stimulatory molecules on the surface of the APC. Thus presentations by dendritic cells or activated macrophages, which express high levels of MHC, class II in addition to co-stimulatory molecules, results in highly effective T cell activation (**Figure-6**). Furthermore, the interaction of CD40L on activated T cells with CD40 on dendritic cells is important for the high level production of IL-12 that is necessary for the generation of an effective TH1 response. However, if the presented antigen to T cells by a 'non-professional' APC that is unable to provide co-stimulation, then the unresponsiveness or immune deviation results. For example, when naïve T cells are exposed to antigen by resting B cells, they fail to respond, and become tolerized. Recent experimental observations illustrate this point. It has been shown that neonatal animals are more susceptible to tolerance induction. Thus mice are resistant to the induction of EAE after administration of MBP in incomplete Freund's adjuvant during the neonatal period. This has been shown to be due to the development of a dominant TH2 response. As EAE is mediated by a TH1 response, the prior TH2 response to MBP prevents the development of the pathological response.

Adjuvants may filtrates immune responses by inducing expression of high levels of MHC and co-stimulatory molecules on APCs. Their ability to activate Langerhans' cells furthermore leads to migration of these skin dendritic cells to the local draining lymph node where effective T cell activation can occur. The importance of the dendritic cell in initiating a cytotoxic T lymphocyte (CTL) response is illustrated by experiments showing that new-born female mice injected with male spleen cells fail to develop a CTL response to the male antigen, H-Y. However if male dendritic cells are injected into female new-born mice, a good H-Y-specific CTL response develops.

B. Regulation by Antibodies

1. Blocking the antigenic determinants.
2. Antibody-feed back mechanism (increased ab affinity).

Antibody has been shown to exert feedback control on the immune response. Passive administration of IgM antibody together with an antigen specifically enhances the immune response to that antigen, whereas IgG antibody suppresses the response. This was originally shown with polyclonal antibodies, but has since been confirmed using monoclonal antibodies (**Figure-4**).
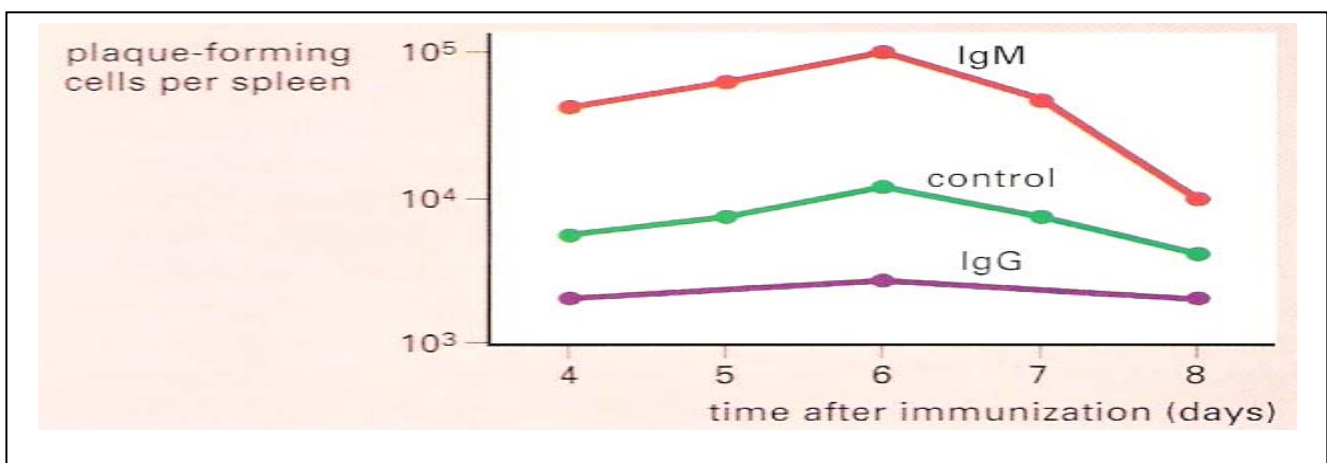
Feedback control by antibody

Figure-4 Mice received a monoclonal IgM anti-SRBC (sheep red blood cells), IgG anti-SRBC or medium alone (control). Two hours later, all groups were immunized with SRBC. The antibody response, measured over the following 8 days, was enhanced by IgM and suppressed by IgG.

The ability of passively administered antibody to enhance or suppress the immune response has certain clinical consequences and applications:

- Certain vaccines (e.g. mumps and measles) are not generally given to infants before 1 year of age. This is because levels of maternally derived IgG remain high for at least 6 months after birth; the presence of such passively acquired IgG at the time of vaccination would result in the development of an inadequate immune response in the baby.

- In case of Rhesus (Rh) incompatibility, the administration of anti-RhD antibody to Rh$^-$ mothers prevents primary sensitization by fetally derived Rh$^+$ blood cells, presumably by removing the foreign antigen (fetal erythrocytes) from the maternal circulation.

The mechanisms by which antibody modulates the immune response are not completely defined. In the case of IgM enhancing plaque-forming cells, there are thought to be tow possible interpretations:

- IgM-containing immune complexes are taken up by Fc receptors or C3 receptors on APCs and are processed more efficiently than antigen alone.

- IgM-containing immune complexes stimulate an anti-idiotypic response to the IgM, which amplifies the immune response. (Idiotypic regulation is discussed below).

IgG antibody can suppress specific IgG synthesis

For IgG-mediated suppression there are also various ways in which the antibody is known to act:

Antibody blocking – passively administered antibody binds antigen in competition with B cell (fig.11.5). The impact of the IgG in this case is highly dependent on the concentration of the antibody, and on its affinity for the antigen compared with the affinity of the B-cell receptors. Only high-affinity B cells compete successfully for the antigen. This mechanism is independent of the Fc portion of the antibody.

Receptor cross-linking – IgG antibody is also known to have an effect that is Fc dependent. Experiments have demonstrated that immunoglobulin can inhibit B-cell differentiation by cross-linking the antigen receptor with the Fc receptor (FcγRIIb) on the same cell (**Figure-5**). In this case, the antibodies may recognize different epitopes.

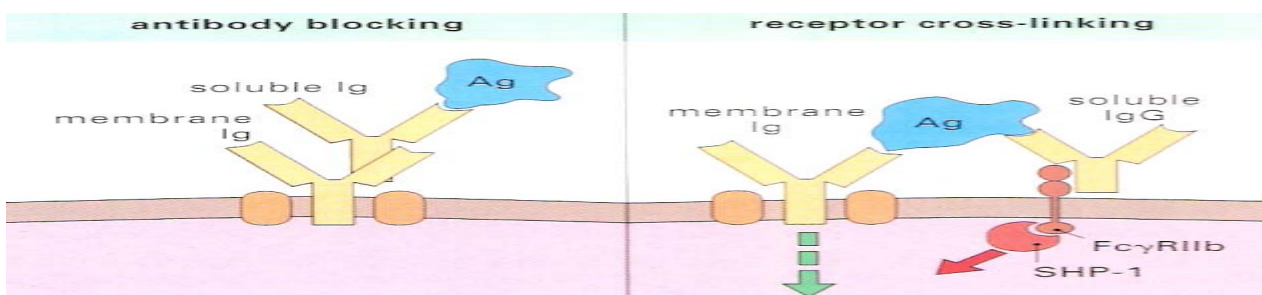## Antibody-dependent B-cell suppression

Figure-5 Antibody blocking: High doses of soluble Ig block the interaction between an antigenic determinant (epitope) and membrane immunoglobulin on B cells. The B cell is then effectively unable to recognize the antigen. This receptor-blocking mechanism also prevents B cell priming, but only antibodies which bind to the same epitope to which the B cell's receptors bind can do this. Receptor cross-linking: Low doses of antibody allow cross-linking by antigen of a B cell's Fc receptors and its antigen receptors. The FcγRIIb receptor associates with a tyrosine phosphatase (SHP-1) which interferes with cell activation by tyrosine kinases associated with the antigen receptor. This allows B cell priming, but inhibits antibody synthesis. Antibodies against different epitopes on the antigen can all act by this mechanism.

Doses of IgG that are insufficient to inhibit the production of antibodies completely have the effect of increasing the average antibody affinity; this is because only those B cells with high-affinity receptors can successfully compete with the passively acquired antibody for antigen. For this reason, antibody feedback is believed to be an important factor driving the process of affinity maturation (**Figure-6**).
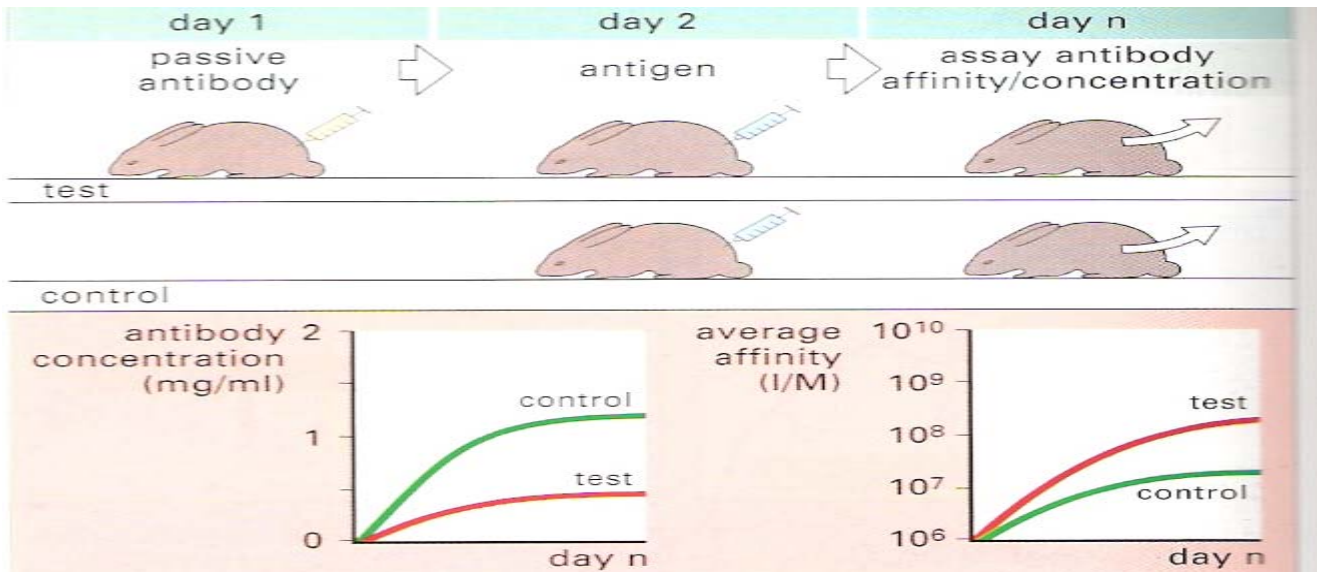
Antibody feedback on affinity maturation

Figure-6 The effect of passive antibody on the affinity and concentration of secreted antibody. One of two rabbits was injected with antibody (passive antibody) on day 1. Both rabbits were immunized with antigen on day 2 and the affinity and concentration of antibody raised to this antigen were assayed at a later time (day n). The antibody assay results show that passive antibody reduces the concentration, but increases the affinity of antibody produced.

C. Regulation by Immune Complexes

Immune complexes may enhance or suppress immune responses

One of the ways in which antibody (either IgM or IgG) might act to modulate the immune response involves an Fc-development mechanism ad immune-complex formation with antigen. Immune complexes can inhibit or augment the immune response (**Figure-7**). By activating complement, immune complexes may become localized via interaction with CR2 on follicular dendritic cells. This could facilitate the immune response by maintaining as source of antigen. CR2 is also expressed on B cells and, as co-ligation of CR2 with membrane IgM has been shown to activate B cells, immune complex interaction with CR2 of the B-cell-co-receptor complex and membrane Ig might lead to an enhanced specific immune response.

The immune response of patients with malignant tumors is often depressed, and it has been postulated that this is the result of the presence of circulating immune complexes composed of antibody and tumor cell antigens.

1. Inhibition: B-cells Fc receptors are crossed-linked to its ag receptor, it will inhibits the signals of B-cell activation.
2. Augmentation: When the immune complex is presented to B-cells via APC.
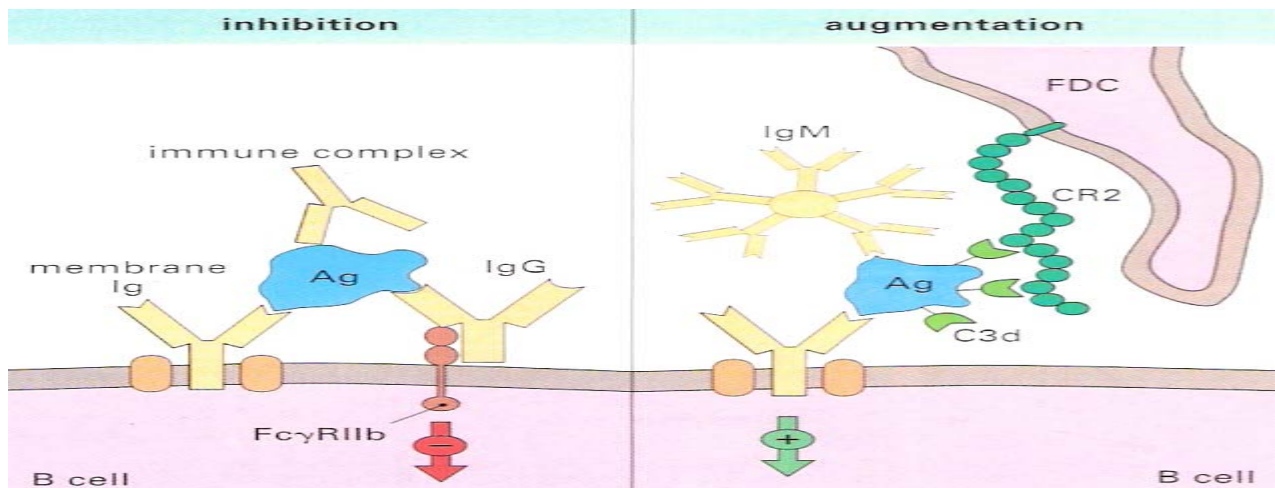
Regulatory effects of immune complexes

Figure-7 Immune complexes can act either to inhibit or to augment an immune response. Inhibition: When the Fc receptor of the B cell is cross-linked to its antigen receptor by an antigen-antibody complex, a signal is delivered to the B cell, inhibiting it from entering the antibody production phase. Passive IgG may have this effect. Augmentation: Antibody encourages presentation of antigen to B cells when it is present on an antigen-presenting cell (APC), bound via Fc receptors or, in this case, complements receptors (CR2) on a follicular dendritic cell (FDC). Passive IgM may have this effect.

D. REGULATION BY LYMPHOCYTES

T-cells clearly modulate the immune response in a positive sense by providing T-cell help. Furthermore, the kind of help which is generated (TH1 or TH2) affects the nature of the immune response, favoring either humoral or cell-mediated immunity. In addition, there is clear evidence that T cells are capable of down-regulating immune responses (**Figure-8**). Apart from the potential for TH1 and TH2 cells to regulate the immune response through an immune deviation, there is now good evidence for two further CD4$^+$ T cell subsets, Tr1 and TH3, which in cytokine production; Treg or Tr1 cells make IL-10, while TH3 cells secrete high levels of TGFβ but lower levels of IL-4 and IL-10.

CD4$^+$ T cells can prevent the induction of autoimmunity

It has been shown in many experimental models of autoimmune disease that CD4$^+$ T cells can prevent the onset of disease. As mentioned previously, the route of antigen administration and therefore the initial APC together with the nature of the antigen influence the outcome of an immune response. For example, the administration of high doses of autoantigen (often given in a soluble or de-aggregated form) prevents induction of autoimmunity. Comparable observations have been made in other experimental autoimmune conditions if the antigen is given orally or as an aerosol.

This inhibition has been shown to be due to CD$^+$ T cells which, in the case of experimentally induced autoimmune thyroid disease, have been shown to prevent both the development of autoimmune thyroiditis and autoantibodies to thyroglobulin (**Figure-9**). This ability of the CD4$^+$ T cells to inhibit both autoimmune thyroiditis and autoantibody production irrespective of isotype shows that the suppression to indeed autoimmunity is not attributable to an immune deviation; that is a switch from a TH1 response to a TH2 response.

Administration of a non-depleting anti-CD4 antibody at the same time as an immunogenic dose of thyroglobulin not only prevents the development of autoimmunity, but also results in the development of a population of CD4$^+$ T cells that can transfer specific tolerance to native recipients (fig.11.10). The exact mechanism by which these T cells exert such a negative influence is not entirely clear but recent experimental results suggest an involvement of TGFβ and IL-10 in this suppression.

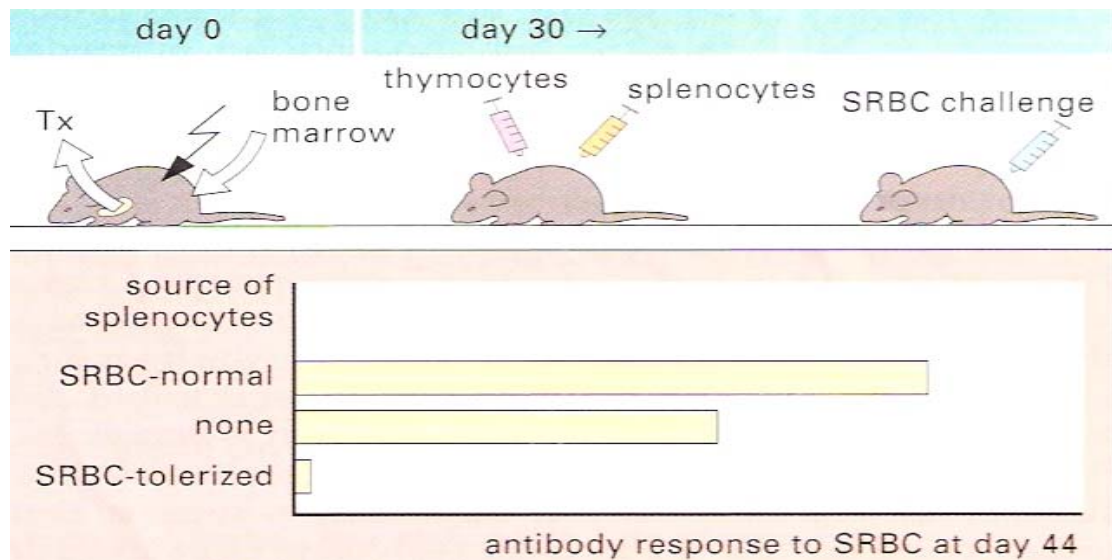# Suppressor cells in immunological tolerance

Figure-8 Thymectomized and irradiated mice were reconstituted with bone marrow cells. After 30 days they were decolonized with thymocytes and splenocytes, and challenged with sheep red blood cells (SRBC). At day 44, recipients given splenocytes primed with immunogenic doses of SRBC had made a strong response. Animals receiving no spleen cells had moderate response. Animals receiving cells from mice tolerized to SRCB (with a high dose of antigen) did not respond, indicating that cells from tolerized animals had actively suppressed the response in the recipient.

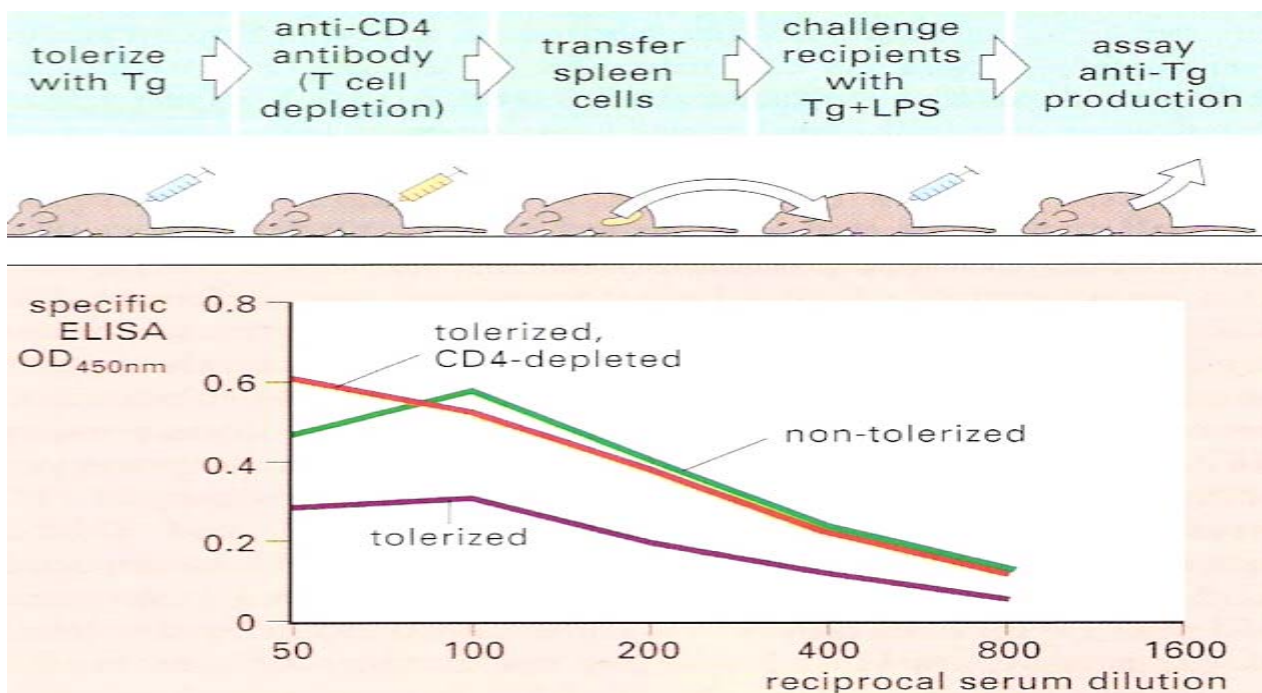# Transfer of tolerance by CD$^+$ T cells

Figure-9 Mice were injected intravenously with 200 μg of mouse thyroglobulin (Tg) to induce tolerance. (A control group was not tolerized). Part of the tolerized group was further treated with *in vivo* with depleting anti-CD4 antibodies, to remove CD4$^+$ T cells. For each mouse in each of these three groups (non-tolerized; tolerized; tolerized and CD4-depleted), spleen cells were transferred into an irradiated syngeneic recipient. The recipients were then challenged with mouse thyroglobulin and LPS, and their anti-Tg antibody response was assayed using ELISA. Anti-CD4 treatment removed the ability to transfer tolerance.

Tн cell subsets are involved in the regulation of immunoglobulin production

The production of different cytokines by different TH (CD4$^+$) lymphocyte subpopulations probably provides an explanation for certain observations regarding the regulation of IgE synthesis. Cross-regulation of TH subsets has been demonstrated whereby cytokines such as interferon-γ (IFNγ), secreted by TH1 cells, can inhibit the responsiveness of TH2 cells. In addition, IL-10 produces by TH2 cells down-regulates B7 and IL-12 expression by APCs, which in turn inhibits TH1 activation. IL-12 is important in the development TH1 responses and the TH1/TH2 balance is modulated both by the level of expression of IL-12 and by expression of the IL-12 receptor. The high affinity IL-12R is composed of two chains, β1 and β2, with both chains being expressed only in TH1 cells. Both TH1 and TH2 cells express the β1 chain and expression of the β2 chain is induced by IFNγ and inhibited by IL-4. T cell subset development has also been shown to be influenced by IFNα, which favours TH1 development, even in the presence of IL-4 and neutralization of IL-12. Thus the preferential activation of TH1 or TH2 cells may result in an immune deviation – the selection of a particular type of effector response. The selective biasing of response may prove useful in the treatment of autoimmune diseases and of allergy.

CD8$^+$ T cells can transfer resistance and tolerance

CD$^+$ T cells have also been shown to regulate immune response. CD8$^+$ T cells have been found in the spleens of animals tolerized to MBP by oral dosing of the antigen. These cells can adoptively transfer resistance to EAE *in vivo*. The T cells not only suppress T cell responses to MBP *in vitro*, but also perform bystander suppression of other unrelated autoantigens in the brain. This effect is thought to be mediated by TGFβ.

E. Regulation of the immune response by CD4$^+$ T is a normal physiological process

The role of such CD4$^+$ or CD8$^+$ T cells mediated regulatory effects in normal physiology has been questioned. However, the observation that CD4$^+$ T cells which are able to prevent autoimmunity are present in unmanipulated normal animals supports their importance in homeostasis. Peripheral T cell lymphopenia can lead to the development of autoimmune disease. Experiments suggest that a subpopulation of T-helper cells expressing high levels of CD25 and low levels of CD45RB play a role in regulating the immune responses and maintaining peripheral tolerance to self antigens. For example it has been shown that CD25$^+$CD4$^+$CD45RB^{Lo} expressing cells are able to regulate colitis. Transfer of CD4$^+$CD45RB^{Hi} cells alone into severe combined immune deficient (SCID) mice causes colitis whereas co-transfer of CD4$^+$CD45RB^{Lo} cells prevents the development of colitis in recipient SCID mice. Prevention of colitis by these cells is in part due to IL-10 production (fig.11.12). This role for IL-10 in the regulation of inflammatory bowel disease (IBD) is supported by the observation that treatment of recipient mice with anti-IL-10R antibody abrogates the ability of CD4$^+$CD45RB^{Lo} cells to prevent the development of colitis. These observations were subsequently confirmed using IL-10 knockout mice, which failed to control disease. This T cell population which regulates the development of IBD by production of IL-10 has been termed a Treg cell or Tr1 cell. TGFβ is required for the development of these regulatory T cells. Recent experiments indicate that Treg cells constitutively express CTLA-4, a molecule associated with negative regulation of T-cell activation.

The spontaneous development of IBD in IL-10 knockout and TGFβ knockout mice is consistent with a role for these cytokines in the function of the regulatory T cells. Furthermore, the spontaneous development of multi-organ infiltrates and uncontrolled lymphocyte activation in CTLA-4 knockout mice supports a key role for this molecule in immunoregulation.

F. REGULATION BY NK AND NK T-CELLS

Natural killer (NK) cells make cytokines and chemokines and thus play an important role in the innate immune response to infections and tumors. Their production of immunogulatory cytokines and

chemokines at early stages in the immune response influences the characteristics of the subsequent adaptive immune response and thus can influence the outcome of the immune response. These cells play a key role in the early immune response to intracellular pathogens, largely through their production of IFNγ which activates macrophages and facilitates differentiation of TH1 cells. NK cell activity itself is induced by a variety of cytokines including IFNβ, IL-15, IL-18 and IL-12. NK cells in turn are negatively regulated by cytokines such as IL-10 and TGFβ.

In the mouse, NK T cells produce cytokines when their TCR engages glycolipids in association with CD1d. It has been suggested that these cells play an immunoregulatory role in the control of autoimmunity, parasite infection and tumor cell growth. Recent experiments suggest that NK T cells secreting IFNγ are able to induce NK cell activation, increasing both NK proliferation and cytotoxicity. They are known to be capable of making IL-4 and IFNγ. Whether they make IL-4 or IFNγ is dependent on the cytokines present in the microenvironment when they are activated. The presence of IL-7, for example, has been shown to elicit IL-4 production in NK T cells and thus promote a TH2 response. This ability to make IL-4, particularly in the thymus, has been associated with the prevention of autoimmunity. For example, non-obese diabetic (NOD) mice have a deficit in NK T cells, and injection of NK T cells into this mouse strain has been shown to prevent the spontaneous development of autoimmune diabetes.

G. REGULATION BY LOCALIZATION OF CELLS

The spatial and temporal production of chemokines by different cell types is an important mechanism of immune regulation. There is god evidence to suggest that recruitment of TH1 and TH2 cells is differentially controlled thus ensuring the maintenance of locally polarized immune responses. The expression of different chemokine receptors on TH1 cells (CXCR3 and CCR5) and TH2 cells (CCR3, CCR4, CCR8) allows chemotactic signals to produce the differential localization of T cell subsets to sites of inflammation (**Figure-7**). Since chemokines can be induced by cytokines released at sites of inflammation, this provides a mechanism for local reinforcement of particular types of response. Once a response is established the T cells can induce the further migration of appropriate effector cells. This is clearly illustrated in TH1 responses where the secondary production of MCP-1, MIP-1α, IP-10 and RANTES serves to focus mononuclear phagocytes to the area of inflammation. The ability of cytokines such as TGFβ, IL-12 and IL-4 to influence chemokine or chemokine receptor expression provides a further level of control on cell migration or recruitment.

Several viruses have been shown to evade the host immune response by interfering with the chemokine/chemokine receptor interactions which are pivotal for an effective inflammatory response. They do this by making chemokine receptor antagonists or chemokine receptor homologues which serve to either blockade chemokine receptors or neutralize chemokine activity.

Immune responses do not normally occur at certain sites in the body such as the anterior chamber of the eye and the testes. These sites are called immune privileged. The failure to evoke immune responses in these sites is partly due to the presence of inhibitory cytokines such as TGFβ and IL-10 which will inhibit inflammatory responses. The presence of migration inhibition factor (MIF) in the anterior chamber of the eye would furthermore inhibit NK activity. The constitutive expression of FasL in cells of the testes and the eye has additionally been proposed as a means of eliminating Fas-expressing lymphocytes that reach these sites, through apoptosis

H. IDIOTYPIC MODULATION OF RESPONSES

Tolerance to self antigens is established during ontogeny. However during the neonatal period the unique binding regions of antigen specific receptors on B and T cells are present at levels that are too low to generate tolerance. Similarly, although antibodies are present in the serum, tolerance only develops to their Fc portions because only these are present in sufficient concentration; tolerance does

not develop to the unique determinants in the heavy and light chains that determine antigen-binding specificity. Individual T cell receptors and immunoglobulins are therefore immunogenic by virtue of these unique sequences, known as idiotypes. Antibodies formed against these antigen-binding sites are called anti-idiotypic antibodies, and are capable of influencing the outcome of an immune response.

Idiotypic determinants may be encoded in the germ line V-region genes, or they may be generated by the process of recombination and mutation involved in producing functional V-region elements. Immunogenic epitopes in or around the binding sites are termed idiotope. Jerne proposed that an immune network existed within the body which interacted by means of idiotype recognition. According to this proposition, when an antibody response is induced by antigens, this antibody will in turn evoke an anti-idiotypic response to itself. This hypothesis is conceptually interesting, but the role of such an idiotype network in controlling a normal immune response remains unclear.

Idiotype interactions may enhance or suppress antibody responses

There is good evidence that anti-idiotypes can affect the representation of recognized idiotypes in an immune response. For example, when C57B1/6 strain mice are challenged with the hapten, nitophenyl (NP), they produce antibodies that are largely restricted to a few defined idiotypes, for example the idiotype 146. Anti-idiotype to this antibody (idiotype 146) can enhance or suppress the production of idiotype 146 when the mice are subsequently challenged with NP on a carrier protein. The observed effect depends on the amount of anti-idiotype given and is idiotype specific, as the overall level of anti-NP antibody is hardly affected. Most importantly, the amounts of anti-idiotype used are within the normal physiological range for particular idiotype-bearing antibodies, which suggests that idiotypic regulation may occur *in vivo*. This kind of observation has been made in other idiotypic systems.

Dramatic effects are observed when anit-idiotype is administered neonatally, when the effect may be life long. For example, the ability of neonatal mice to mount an anti-phosphoryl choline response is greatly reduced after being injected with anti-idiotype to T15 (T15 is a major idiotype in the response to phosphoryle choline). The reduction lasts many months. The response which these mice subsequently make is dominated by non-T15 immunoglobulins.

I. NEUROENDOCRINE MODULATION OF IMMUNE RESPONSES

It has long been known that stressful conditions may lead to a suppression of immune functions, for example reducing the ability to recover from infection. There is considerable evidence demonstrating that the nervous, endocrine and immune systems are interconnected. Broadly, there are tow main routes by which events occurring in the CNS could module immune function:

- Most lymphoid tissues receive direct sympathetic innervation, both to the blood vessels passing through the tissues, and directly to the lymphocytes themselves.

- The nervous system directly or indirectly controls the output of various hormones, in particular corticosteroids, growth hormone, prolactin, α-MSH, thyroxine and adrenaline.

Lymphocytes express receptors for many hormones, neurotransmitters and neuropeptides, including ones for steroids, catecholamines (adrenaline and noradrenaline), enkephalins, endorphins, substance P and vasoactive intestinal peptide (VIP). Expression and responsiveness vary between defferent lymphocyte and monocyte populations, such the effect of different transmittes may vary in different circumstances. However, one particularly important control is mediated by corticosteroids, endorphins and enkephalins, all of which may be released during stress, and all of which are immunosuppressive *in vivo*. The precise in vitro effects of endorphins vary greatly, depending on the system and on the doses used; some levels are suppressive, and others enhance immune functions. It is certain, however, that the corticosteroids act as a major feedback control on immune responses. It has been found that lymphocytes themselves can respond to corticotrophin releasing factor to generate their own ACTH, which in turn induces corticosteroid release.

Corticosteroids have been shown to inhibit TH1cytokine production while sparing TH2 responses. Corticosteroids to the production if IL-1 by macrophages and of IL-6 by T cells, both IL-1 and IL-6 are synthesized by neurons and glial cells and, in addition, by cells in the primary and adrenal glands, further emphasizing their potential as bidirectional mediators in response to stress.

J. GENETIC CONTROL OF IMMUNE RESPONSES

Familial patterns of susceptibility to infectious agents have suggested that resistance or susceptibility might be an inherited characteristic. Such patterns of resistance and susceptibility are also shown with autoimmune diseases. Often many genes are involved in governing susceptibility or resistance to disease and the disease is thus said to be under polygenic control. Considerable advances have been made in mapping and in some cases identifying the genes governing the response to some of the diseases. This has been largely due to the development and use of techniques such as micro-satellite mapping and the availability of a large number of DNA samples from susceptible and resistant individuals. In most cases these studies have led to the identification of potential candidate genes but their real role in disease susceptibility remains to be clarified. In other cases single mutations in genes of known function have been found and the mechanism by which they contribute to disease identified.

There are several ways in which genes influence the immune response.

MHC haplotypes influences the ability to respond to an antigen

With the development of inbred mouse strains, it become possible to analyze genetic influences more rigorously and it was conclusively demonstrated that genetic factors have a role in determining immune responsiveness. For example, strains of mice with different MHC haplotypes vary in their ability to mount an antibody response to specific antigens. This function depends on MHC class II molecules, and is specific for each antigen – a high-responder strain for some antigens will be a low-responder strain for others. It was shown furthermore that genes within the MHC play a fundamental p;art in influencing the response against infectious agents.

MHC-linked immune response genes control all immune responses that involve antigen recognition by T cells

As discussed in previous chapters, the immune response depends upon the activation of clones of lymphocytes. In the case of T cells, these recognize antigen only when it is presented to them as peptide complexes to class I or class II major histocompatibility (MHC) antigens. For example, $CD8^+$ T-cytotoxic (Tc) cells will only lyse virally infected target cells derived from an MHC class I-matched mouse strain. Genetic restriction can be tracked to specific MHC molecules from one particular locus. For example, in the cytotoxic response of A.TL mice to LCMV virus, the cytotoxic T cells are principally directed against H-2D locus targets. However, Sendai virus in this strain is presented more effectively by other MHC class I molecules; MHC restricted recognition is learnt in the thymus during ontogeny.

As will be explained in Chapter 12, T cells are subjected to two selection process during development in the thymus – positive and negative selection. The peripheral T-cell repertoire is influenced both by the range of self antigens presented in the thymus and in the periphery, and by their ability to bind to the individual's MHC antigens). The ability of peptide to bind to MHC is determined by the amino acid sequences in the binding sites of the MHC molecules. We now know that most of the polymorphic residues in MHC molecules reside in the peptide-binding groove. Thus the extensive sequence polymorphism of MHC molecules has a deep impact on peptide binding and, as a consequence, on T cell activation.

MHC-linked genes control the response to infections

MHC-linked genes have been shown to play a part in the immune response to infectious agents. In some cases the gene involved is the MHC gene itself, but in others it is believed to be a gene that is simply linked to the MHC.

Susceptibility to infection by Trichinella spiralis is affected by the I-E locus in mice

The first observation that genes (*Ts-1 and Ts-2*) within the MHC could influence the response to parasites involved the susceptibility to *Trichinella spiralis*. (It is interesting that such an effect should be noted with an antigenically complex organism, especially as these parasites express different antigens at different stages in their life cycle, with different APCs being involved in their presentation.) If different recombinant mouse strains are infected with *T. spiralis*, it can be seen that resistance or susceptibility is affected by the I-E locus. Mouse strains that express I-E appear to be susceptible. An additional MHC-linked gene has been shown to influence the response to *T. spiralis*, in this case it is not an MHC-encoded gene, but another gene in liniage disequilibrium. This gene, which has been designed *Ts-2*, maps close to the TNF genes.

The I-E locus also influences susceptibility to Leishmania donvani

Using H-2 congenic mice, it was shown that I-E expressing mice were unable to combat visceral leishmaniasis. Direct involvement of the I-E product in this susceptibility was shown by the ability of anti-I-E antibody, but not the anti-I-E antibody, to enhance parasite clearance. Furthermore, insertion of an I-E transgene into a mouse strain lacing I-E makes them unable to clear parasites from the liver and spleen as effectively as the original strain.

Certain HLA haplotypes confer protection from infection

In humans, a comparison of the HLA haplotypes reveal that certain MHC class I and class II alleles (HLA-B*5301 and DRB1*1302, respectively) were associated with a reduced risk of severe malaria. DRB1*1302 has been shown to bind peptides different form those bound by DRB1*1301, as a result of s single amino acid difference in the β chain. This would clearly influence the response to the malaria parasite. HLA-DRB1*1302 has also been associated with an increased clearance of the hepatitis B virus and hence a decreased risk of chronic liver disease.

In human T-lymphotropic virus-1(HTLV-1) infection, the MHC class I type, HLA-A*02, is associated with a reduction in the risk of disease development. The viral load was lower in HLA-A*02 positive healthy carriers of HTLV-1 correlating wit the presence of high levels of virus-specific cytotoxic T cells. In HIV-1 infection a selective advantage against disease has been noted in individuals expressing maximal HLA heterozygosity of class I loci (A, B and C) and lacing expressing of HLA-B*35 and HLA-Cw*04).

Protection is not necessarily related the class I and II molecules. For example, tumor necrosis factor-α (TNFα) lies within the MHC and polymorphisms in the promoter region of this gene influence its level of expression possibly through altered binding of the transcription factor, OCT-1.One of these polymorphisms, which is commonly associated with cerebral malaria, results in high levels of TNF expression which may lead to upregulation of ICAM-1 on vascular endothelium and to increased adherence of infected erythrocytes and subsequent blockage of blood flow. This polymorphism in the TNFα promoter has also been associated with lepromatous that not tuberculous leprosy and with mucocutaneous leishmaniasis and death from meningococcal disease.

MHC genes have a major influence on susceptibility to autoimmune diseases

Insulin-dependent diabetes mellitus (IDDM), an autoimmune disease in which the bête cells of the pancreas are destroyed by cells of the immune system, is associated with HLA-DR3 and HLA-DR4. The highest risk is in fact seen in HLA-DR3/4 heterozygotes. Because of linkage disequilibrium, although the original associations were seen with DR, they are in reality with DQ. Molecular genetic analysis has permitted the association to be analyzed in more detail, and it seems that the primary association in Caucasians is with DQB1*0302. In multiple sclerosis the initial association with HLA-DR2 in Northern Europeans appears to relate to the extended haplotype DQB1*0602-DQA1*0102-DRB1*1501. Interestingly however there are different risk haplotypes in Southern Europeans. This suggests that different environmental risk factors interact with particular MHC molecules in each population. In rheumatoid arthritis, the predominant association with HLA-DR4 or DR1 in several ethnic groups, but there is little association with HLA-DQ. Figure 11.21 provides examples of MHC linkages to autoimmune disease. The way in which theses MHC association contribute to disease susceptibility remains unclear, but possible explanations include repertoire differences through positive and negative selection on different class II gene, or preferential binding of disease-inducing epitopes of bacteria or viruses to particular MHC molecules. Analysis of the amino acid sequences of peptide binding grooves of HLA-DR4 and DR1 has supported this hypothesis by demonstrating the presence of differently charged residues in susceptible or resistant subtypes of HLA-DR.

The NOD mouse spontaneously develops IDDM and as in humans the development of this autoimmune disease is under polygenic control, with MHC-linked genes playing a major role in determining resistance or susceptibility.

This mouse strain does not express an H-2E molecule and expresses an unusual H-2A heterodimer, I-A^{g7}, comprising Aα^d and Aβ^{g7}. While most mouse H-2Aβ chains have an aspartate at position 57 and a proline at position 56, the NOD H-2β chain contains a serine and a histidine at these respective positions. Transgenic NOD mice expressing mutated H-2A^{g7} where the *Abg7* gene has been mutate to encode an aspartate at position 57 or a proline at position 56 have markedly reduced incidence of IDDM. Additionally restoration of H-2E expression through transgenesis is also prevents IDDM. The crystal structure of this diabetes-associated class II molecule has recently been solved. This shows clearly that peptide binding preferences are different between NOD class II molecules and those of many other strains. Such a difference in peptide binding would have profound implications for central tolerance and peripheral T cell activation.

The ability of H-2E to influence the development of autoimmunity is not just restricted to IDDM. Alternations in levels of H-2E expression either through transgenesis or through use of recombinant mouse strains has been shown to reduce the incidence of SLE-like disease. This effect of H-2E expression has been attributed to an excessive generation of H-2Eα peptides which compete with self peptides for binding to H-2A.

Genes in linkage disequilibrium with MHC influence the development of autoimmunity

NZB x NZW F1 mice spontaneously develop systemic lupus erythematosus (SLE). Disease development is under complex genetic control but one gene has been linked to the H-2^Z of the NZW parent. It has been clearly demonstrated that this association was not with an MHC gene itself, but with the closely linked *Tnfa* gene. The NZW *Tnfa* allele gives rise to the production of low amounts of TNFα. If the concentration of this cytokine is increased, the mice are protected from the development of lupus nephritis.

Association with genes involved in processing

Other MHCp-linked genes have recently been identified which may influence immune responses. These genes are involved in the generation (by proteolysis) and transport of antigen peptide fragments. They are polymorphic, and such polymorphism has functional consequences. For example, in the rat,

different allelic forms of the *cim* locus (encoding TAP2 protein) affect peptide loading into the class I MHC, which in turn affects the ability of the class I MHC molecule to be recognized as an alloantigen. It is therefore possible that some of the MHC-linked disease association that have been identified are attributable to similar genes, involved in proteolysis and transport of antigen peptides to the MHC molecules for presentation to cells of the immune system.

Many non-MHC genes also modulate immune responses

The immune response is also governed by some genes outside the MHC region. However, these genes are generally less polymorphic than MHC genes and they make a lesser contribution to variations in disease susceptibility in a population than do the MHC genes. Nevertheless, their effects have been clearly shown in autoimmune diseases, allergy and infection. For example:

- Individuals with defects in the complement components C1q, C1r, C1s are predisposed to develop SLE and lupus nephritis. Deficiency in C3 leads to an increased susceptibility to bacterial infections and a predisposition to immune-complex disease as does deficiency in C2 and C4, both of which are located within the MHC region. The development of SLE-like symptoms in C1q knockout mice parallels the human situation.

- High IgE production in some allergy-prone families has been shown to associate with the presence of an 'atopy gene' on human chromosome 11q.

- Biozzi generated two lines of mice by selective inbreeding, based on their responsiveness to erythrocyte antigens. These high-responder and low-responder Biozzi mice make quantitatively different amounts of antibody in response to antigenic challenge. The basis for these differences has in part been attributed to genetic differences in macrophage activity. These high- and low-responder strains also differ markedly in their ability to respond to parasitic infections, and this does not necessarily correlate with the amount of antibody they make.

Non-MHC-linked genes affect susceptibility to infection

Macrophages have a key role in the immune system. Genes regulating their activity may therefore determine the outcome of many immune responses. A good example of such genetic control of macrophage function is provided by the *Lsh/Ity/Bcg* gene. This gene governs the early response to infection with *Leishmania donovani, Salmonella typhimurium, Mycobacterium bovis, M. lepraemurium* and *M. intracellulare.* Its influence is on the early phase of macrophage priming and activation, and it has wide-ranging effects, including:

● Up-regulation of the oxidative burst

● Enhanced tumoricidal activity

● Enhanced antimicrobial activity

● Up-regulation of MHC class II expression

Recent congenic studies have identified the natural resistance-associated macrophage protein 1 (*Nrampl*) as the *beg* gene. As *Nramp1* encodes a membrane protein with homology to known transport proteins, the suggestion has been made that it may be implicated in the transport of NO_2^- into the phagolysosome, thus facilitating the killing of intracellular organisms. The human homologue (*NRAMP1*) of the mouse gene, *Nramp1*, has been cloned and several different alleles identified. Polymorphisms in this gene may contribute to resistance to tuberculosis in human although the data thus far is not so convincing as in the mouse.

Polymorphisms in the genes encoding cytokine receptors have been shown to correlate with an increased susceptibility to infection, severe combined immunodeficiency (SCID) or inflammatory conditions. The outcome of the mutation is dependent on the cytokine gene which is affected. For example, humans with mutations in the IL-7Rdevelop a selective deficit in T cells and those with deficiency in the common cytokine receptor γ chain (γc), which is a component of the functional receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 have reduced numbers of T cells and NK cells and have impaired B cell function, in part attributable to the lack of T cell help. Further examples are the mutations in the IFN-γR or IL-12R which markedly increase susceptibility to mycobacterial infection. Lists of genetic defect which contribute to impaired immune responses are listed in.

Mutations in the cytokine promoters have been shown to influence the levels of expression of cytokine. Polymorphisms such as these have been linked to certain autoimmune conditions and also to susceptibility to infections. Susceptibility to severe malaria is under complex genetic control with other genes in addition to MHC playing an important role. Recent studies, for example, have linked the development of cerebral malaria to a polymorphism in the promoter region of the TNFα gene. Other studies have implicated polymorphisms in the promoter region of the inducible NO synthase gene (*NOS2*).

Eosinophils have an important role in the host response to parasitic infection. It has been shown that the degree of eosinophilia following infection is genetically determined, with marked differences seen in different inbred strains of mice. Similar observations have been made in guinea pigs and sheep, in which a consistent correlation has been found between resistance to nematode infection and the extent of eosinophilia. These observations may relate to polymorphisms which influence relevant cytokine and chemokine levels, including IL-5 and eotaxin.

Some genes involved in immune responses can affect disease susceptibility, but not because they affect immune responsiveness. For example, disease progression to AIDS has been shown to be associated with polymorphisms in the chemokine receptor gene-5 (CCR-5). CCR-5 is a co-receptor which is used in the entry of macrophage-trophic strains of HIV-1 into cells. A mutation which inactivates this receptor is found in some individuals of European origin but is rare in populations of Asian or sub-Saharan African descent. Individuals homozygous for this CCR-5 mutation have been found to be very resistant to HIV-1 infection. In this case resistance is related to the reduced primary spread of the virus, rather than an enhanced immune response against it.

Non-MHC-linked genes also affect development of autoimmune disease

Major advances have recently been made in mapping the loci which govern susceptibility to the autoimmune disease, insulin-dependent diabetes mellitus (IDDM). This work has been lartely carried out using the NOD mouse strain, which spontaneously develops an autoimmune disease similar to IDDM in humans. At least 18 genetic loci have been identified in the NOD mouse (*idd-1 to 18*). Only one locus (*Idd-1*) is linked to the mouse MHC on chromosome 17, and is believed to encode MHC class II molecules themselves. The other identity and functional roles in determining resistance or susceptibility are not yet known.

When the lymphoproliferative (*lpr*) gene is present in mouse strains it causes the development of a characteristic clinical syndrome. The mice develop anti-DNA antibodies, rheumatoid factor, circulating immune complexes and glomerulonephritis. There is also a lymphadenopathy in these mice, involving a polyclonal expansion of double negative (CD4$^-$ CD8$^-$) T cells in the periphery. It has been shown that mice with the lpr gene have a defect in CD95 (Fas), a transmembrane molecule belonging to the TNF receptor superfamily that interacts with CD95L to induce apoptosis. (CD95L is a member of the tumor necrosis-nerve growth factor family). The defect in Fas results in the failure of apoptosis but it does not appear to affect negative selection and the generation of a normal repertoire of mature single positive T cells in the thymus. Evidently Fas is only one of the ligands which mediate apoptosis. It is now proposed that the defect leads to the expansion of double-negative T cells in the periphery and an acceleration of an autoimmune syndrome. The defect in apoptosis also affects B cells – autoreactive B cells accumulate in the periphery.

Other studies have shown that the *gld* gene which encodes a defective FasL results in an autoimmune phenotype similar to that seen in mice homozygous for the *lpr* defect. Thus mice which are homozygous for the gld mutation do not express a functional ligand, have a defect in apoptosis of peripheral B and T cells, and develop autoimmunity. The gld gene is located on chromosome 1 in the mouse and thus provide yet another example of a gene that affects immune function, but which is not MHC linked. Comparable syndromes have been described in humans, which are attributable to defects in Fas activity and function (fig.11.25). Studies of patients with autoimmune lymphoproliferative syndrome (ALPS) have provided insight into the interactions between Fas and FasL. Some of the patients suffering from ALPS have a dominant mutation in the gene encoding Fas. Detailed analysis of the mechanism by which the mutant allele dominantly interferes with apoptosis suggests that Fas is normally found as a trimeric complex at the cell surface. Trimer formation is dependent on a domain within Fas called the pre-ligand assembly domain (PLAD) and Fas molecules with mutation within this region interfere with trimer formation by normal Fas molecules. This results in defective Fas/FasL mediated apoptosis and the development of an autoimmune syndrome in patients with the dominant Fas mutation.

Another example of polymorphism affecting immune responses is seen in FcγRIIb in mice. Recall that FcγRIIb inhibits B-cell activation when it is co-ligated by antibody bound to a multivalent antigen. Polymorphisms have been detected in the FcγRIIb transcriptional regulatory regions of the mouse. One of these polymorphisms which results in diminished expression of FcγRIIb on germinal centre B cells is common in autoimmune prone mouse strains and is associated with elevated levels of Ig consistent with a lack of feedback regulation on the B cell. This has led to the suggestion that such polymorphism may contribute to some forms of autoimmune pathology such as SLE and Sjogren's syndrome.

Clearly, many genes involved in immune responses affect susceptibility to infection and autoimmunity. Gene defects often cause serious impairment to the immune system. Functional polymorphism is particularly evident in MHC class I and II genes, but there is increasing evidence for limited polymorphism in gene structure or gene expression of many non-MHC immune response genes. Cumulatively, these variations may well be as important as those in the MHC-linked genes.

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