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Systemic autoimmune diseases: Possible involvement of superantigens in the abnormal immune response

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Multiple autoantibody responses associated with systemic autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis were once considered to be due to the nonspecific activation of the immune system, possibly by polyclonal B-cell mitogens. But unlike a mitogen-induced response, autoimmune response associated with these diseases was found to be class II major histocompatibility complex (MHC) restricted and T-helper cell regulated. So it is proposed that rather than acting alone, the mitogens may be synergistically acting along with a weak self-antigen to give a specific response. However, recently a group of antigens have been identified, which eliminate the requirement of such a synergistic action. They give antigen-specific and polyclonal antibody responses, similar to the autoimmune response in the above diseases, by employing T-helper cells and class II MHC. These proteins termed 'superantigens' bind to T-helper cells bearing a limited set of β-chain variants of T-cell receptor. Though superantigens require class II MHC molecules for presentation to T cells, the binding is to the non-polymorphic region of MHC unlike the conventional antigens which bind to the polymorphic region. These antigens explain the initiation of autoimmune response by the non-specific activation of T-helper cells.

Autoimmune diseases comprise a group of disorders where there is nothing apparently in common other than an exaggerated immune response to one or more of the self-antigens[1]. Historically autoantibodies were considered to be something abominable and the theory of sequestered antigens and forbidden clones was proposed[2,3]. Recent studies however show that autoantibodies and autoreactive T cells are normal[4–7]. Now the question arises as to what contributes to the diseases.

Autoimmune diseases are classified into organ-specific and systemic diseases[8]. In organ-specific diseases the autoimmune response is directed against some unique antigens present on one or more of the tissues, whereas in systemic autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) the autoimmune response is found to be multiple autoantigens. Since the antibody response in these diseases is not very highly specific, it was considered that the immune response is due to the non-specific activation of the immune system.

Nonspecific immunostimulation

Though antigen-dependent stimulation is the convention, there exist alternate mechanisms for the activation of the immune system. Substances which activate T and B cells in this way are called mitogens[9]. Many carbohydrate-binding proteins (lectins) possess the potency to activate T and B cells. B cells can also be activated by anti-immunoglobulin and B-cell mitogens (lipopolysaccharides, dextran sulphate, etc). Induction of antibodies by these compounds is not specific and results in multiple antibodies including autoantibodies[10,11]. It is speculated that polyclonal B-cell mitogens (PBMs) may be responsible for systemic autoimmune diseases[12]. Administration of multiple immunostimulators in mice brings about severe but transient autoimmune syndrome[13,14]. Furthermore, circulating PBMs have been detected in RA and SLE patients[15,16]. But there are many drawbacks in extending this PBM-induced model to the actual disease. Autoantibodies induced by potent B-cell stimulators like epstein barr virus (EBV) are predominantly lgM (ref. 17) whereas, in these diseases the pathogenic antibodies are of other isotypes. Potent

PBMs bring only transient autoimmunity and the circulating T-suppressor cells (Ts) can control the effect of the PBM[12]. MHC class II association has been shown in many autoimmune diseases[18,19]. Finally, specific lymphocytes have been cloned from SLE which give helper function for the production of anti-DNA antibodies[20]. So rather than acting independently mitogens may activate B cells synergistically along with a weak autoantigen, resulting in the production of specific antibodies[12].

Specific activation of the immune system by antigens

An immunologically primed animal responds to the subsequent exposure of the same antigen by producing effector cells to eliminate them[21]. B-lymphocytes are involved in the antibody production. Thymus derived T-lymphocytes are responsible for the control of immune response. Functionally they belong to T-suppressor (Ts), T-helper (Th) and T-cytotoxic (Tc) cells. Their functions include (a) controlling the B-cell responses by Th and Ts cells, and (b) direct killing of the target cells by Tc cells.

Immune response will be mounted only if the antigen is foreign. Three components are involved in giving a specific response. They are the processed antigenic fragments, class II MHC molecules present on antigen-presenting cells (APC), and the antigen receptors present on T cells (TCR) (Figure 1).

Antigen. It is processed into small fragments by proteolytic enzymes and expressed on the surface of the APC in association with class II MHC. Antibodies will be produced only to regions (epitopes) of the antigen, which do not have any resemblance to the self.

Major histocompatibility complex. Class II MHC antigens are membrane-bound glycoproteins present on the surface of APC, composed of α and β subunits. These are one of the most polymorphic proteins known and bind to a variety of peptides[22]. The processed antigenic peptides associate with the MHC and are recognized by the T cells.

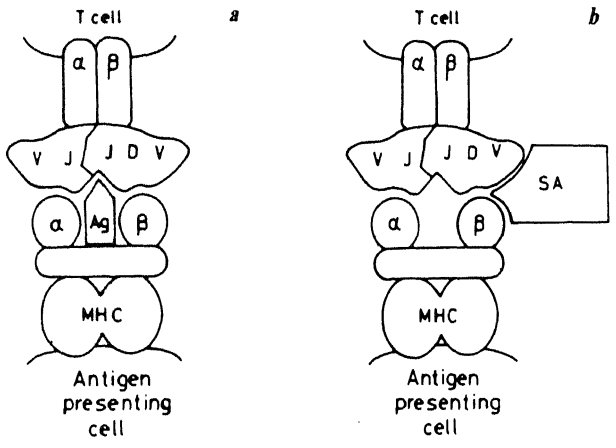

Figure 1. Schematic representation of the trimolecular complex formed of $\alpha\beta$ TCR, class II MHC and ***a***, conventional antigen, ***b***, superantigen (adapted from ref. 33).

T-cell receptors. These are membrane-bound heterodimeric glycoproteins[23,24]. Two kinds of TCRs are reported—$\alpha\beta$ and $\gamma\delta$. $\alpha\beta$ TCRs are involved in the recognition of class II MHC-associated peptides. Similar to immunoglobulin G (IgG), TCR is also assembled by somatic recombination of α, β germ line components from constant (C), variable (V), joining (J) and diversity (D) regions. α-Chain resembles the light chain of IgG in not having a D region and β-chains resemble the heavy chain. Two other molecules are also associated with TCR. The CD_3 complex composed of three peptides is involved in the signal transduction and T_4 molecules (present on Th, Ti) or T_8 molecules (present on Tc, Ts), which are associated with particular subtypes of T cells.

T cells bearing particular receptors interact with the antigen associated with the MHC and secrete lymphokines which control the antibody production and class switching. TCRs present on mature cells recognize only foreign antigens. In this respect, past studies have shown that while inside the thymus T cells undergo a positive selection, where from the myriads of immature T cells, only those TCR-bearing cells will be selected which associate with self class II MHC[25] and a negative selection where self-reactive TCR-bearing T-cells are eliminated[26]. Though this mechanism is not complete this eliminates the self-reactivity to a great extent. Other than this central mechanism, the Ts cells present in the circulation control the autoreactive antibody responses.

Autoimmunity could result because of the hyperactivity of Th cells, deficiency of Ts cells or non-responsiveness of B cells to the T cell signal. In animal models of SLE, (MRL/lpr, BxSB, etc.) one of the above mechanisms has been attributed as the cause of autoantibody production[27,28].

Ts cell deficiency or Th cell hyperactivity alone does not explain the autoantibody production. Environmental factors are also implicated and most possible among them is bacterial or viral antigens with structural resemblance to host (molecular mimicry). Antibodies produced against these proteins cross-react with self-antigens[29]. Recently a group of bacterial antigens have been identified which have very high antigenic potency compared to usual bacterial antigens. Conventional antigens activate 1 in 1000 of the T cells. These bacterial antigens activate higher number of T cells (1 in 5) like a mitogen. To show their comparatively high antigenicity, these antigens are termed as 'superantigens' (SA)[30,31].

Superantigens

Two types of superantigens have been reported, the minor lymphocyte stimulating antigens of mice; and the antigens isolated from Staphylococcus, Streptococcus and Mycoplasma strains[32]. The significance of self-superantigens (endogenous SA) reported from mice is not known.

Three groups of microbial superantigens (exogenous SA) have been reported. They are the enterotoxins of *S. aureus* (SE), pyrogenic toxins of group A Streptococcus (SPE) and the SA from *Mycoplasma arthritides*[33]. Enterotoxins are basic proteins with molecular weight ranging from 20 to 30 Kd. Streptococcal pyrogenic toxins though identical to each other are different from styphylococcal toxins. Mycoplasma-derived superantigen is not well characterized. Superantigens from staphylococcus are encoded by phages[34].

Though earlier considered to be indiscriminate mitogens, recent studies show that SA bring about antigen specific as well as non-specific antibody responses with the involvement of T cells and APC.

Activation of immune system by superantigens

Superantigens stimulate immune system like any other antigens by employing TCR and class II MHC. This is different from the T-cell mitogen-mediated responses where TCR or MHC does not play any role. But superantigen-induced response is polyclonal like mitogen-induced[35] response.

Requirement of TCR

Superantigens do not stimulate all T cells, neither do they have any preference to any functional T-cell subsets. They activate T cells bearing a set of β-chain variants of the TCR irrespective of the α-chain variants[36]. SA binds to the variable region of β-chain of the TCR (Figure 1*b*). This include antigen specific and T cells of other specificities. The nature of the binding is not known. Since all the exogenous superantigens reported are basic proteins, charge-mediated binding cannot be ruled out. Though such binding is not studied in detail in the case of TCR, it has been shown that antibody molecules are capable of heterologous antigenic cross-reactivity mediated by charge interaction[37,38].

Requirement of class II MHC

Studies using particular class II MHC transfected mouse fibroplasts have shown that superantigens though require class II MHC, do not show any specificity to particular isotype or allotype[39]. Subsequently, it was shown[40] that unlike the processed antigenic peptide from conventional antigens which bind to the highly polymorphic regions of the MHC, SA binds to the non-polymorphic region of the MHC outside the antigen-combining site and form ternary complex with TCR and antigen during the T-cell recognition (Figure 1*b*). This recognition results out of the nonspecific binding (MHC unrestricted) but still result in the proliferation of effector T cells. Furthermore, superantigens do not undergo any processing.

Involvement of superantigens in diseases

In view of the high antigenicity and the mode of action, superantigens are likely to be the candidate involved in the process of autoimmunization. Preliminary studies with SLE and RA implicate superantigens as the possible environmental factor responsible for autoimmunity.

Systemic lupus erythematosus

It is one of the most fatal autoimmune diseases mediated by multiple autoantibody responses[41]. Antibodies to dsDNA are unique to SLE. These are implicated in the glomerulonephritis, which brings considerable mortality among SLE patients[42]. Abnormality in the T-cell functions has been reported in SLE[43]. Among the induced models, graft versus host disease (GVHD) represents an intriguing model for the cellular interactions that may give rise to systemic autoimmunity[42]. GVHD results when immune cells from a healthy individual are transferred to an immunologically incompetent host. In this model of autoimmunity the T-helper cells from donors, specific for the recipient class II MHC alloantigens leads to polyclonal activation of the recipient B cell pool, hypergammoglobulinaemia and lymphoid hyperplasia. In selected strains of nonSLE-prone mice this type of an interaction induces a broad spectrum of autoantibodies and immune-complex-mediated tissue damage, similar to SLE[43,44]. This type of abnormal Th–B cell interaction brings about systemic autoimmunity. Any B cells regardless of the specificity or MHC molecule render cognate T cell help by SA-specific T cells.

Tumang *et al.*[35] showed that Th-cell lines specific for micoplasma-derived superantigen (MSA) or staphylococcus-derived toxin-I (SE-I) showed that co-culture of Th-cells with syngenic B cells bearing the SA results in the B-cell proliferation and polyclonal IgG and IgM production. In contrast, antigen-specific (SRBC-specific) B cells are generated only in the presence of the antigen. These findings strengthen the GVHD model and the

role of SA in triggering an autoimmune response in susceptible individuals.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by long-term inflammation of multiple joints[47]. Mononuclear infiltration of the synovial membrane eventually leads to the destruction of the articular cartilage and surrounding structures. Pathogenesis of RA is not known. Several lines of evidence suggest that T cells specific for self-antigens may play a critical role in the initiation of the disease. The linkage of the disease with DR1 and DR4 of the MHC and the findings of sometimes oligoclonally activated $CD4^+$, $\alpha\beta$ T cells in the synovial fluid and tissues of affected joints suggest the involvement of $CD4^+$ and $\alpha\beta$ TCR-bearing class II MHC-restricted T cells in the disease[48]. Partial elimination of these cells leads to amelioration of the disease[49].

While studying the $\alpha\beta$ TCR-mediated response in RA patients, Palliard *et al.*[50] found that the frequency of Vβ14 T cells was significantly higher in the synovial fluid of the affected patients than in the peripheral blood[50]. Vβ-cells are virtually undetectable in the peripheral blood of these patients. β-Chain sequences indicated that one or a few clones dominated the Vβ14 population in the synovial fluid of individual RA patients, where oligoclonality was less marked for other Vβs and for Vβ14 in other types of inflammatory arthritis. These studies implicate Vβ14 cells in the pathology of RA. RA may involve initial activation of Vβ14 TCR-bearing T cells by superantigens and subsequent recruitment of very few activated autoreactive Vβ14 T cell class to the joints while majority of other Vβ14 T cells disappear.

Conclusion

The evidences discussed above are preliminary. Superantigen mediation may not be the sole cause of systemic autoimmunity. Genetic predisposition is equally important. However, the mechanism of action of superantigens gives insight into the process of nonspecific stimulation of the immune system and therapeutic strategy could be devised to treat the patients suffering from autoimmune diseases. For example, in adjuvant-induced arthritis (AA) cytotoxic T cells (A2b) which recognize cartilage proteoglycan bring about the erosion of the joints similar to RA[51]. Lymphocyte vaccination of normals using hydrostatic-pressure treated A2b cells from the above model gives protection against AA. Antiidiotypic T cells are formed which knock out the A2b cells from the circulation and protect the normals from the disease. Similar strategy using antiidiotypic T cells or antibodies to TCR to eliminate SA responsive TCR-bearing Th cells could be applied for the treatment of RA and SLE also.

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RESEARCH ARTICLE

Petrochemistry and tectonic evolution of Munnar granite, Idukki district, Kerala

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Several post-tectonic granite bodies, ranging in age from 740±30 to 512±20 Ma, have been reported from the south-western margin of the South Indian shield. The present study of Munnar granite and the associated rocks has brought out two generations of granite—one, a post-tectonic undeformed non-foliated granite and the other a pre-tectonic regionally deformed, well-foliated gneissic granite. The gneissic granite, showing uniform granite chemistry and mineralogy, is suggested to have been formed by progressive deformation of an older granitoid intrusion. The gneissic granite was earlier considered as part of the migmatite country rock. Thus, two granite-forming events are identified, of which the older granite-event has not hitherto been recorded in the southwestern margin of the South Indian shield.

ACID igneous activity in the Precambrian terrain of Kerala is indicated by several granite bodies (Figure 1). All these granites, falling within the time span of 740±30 Ma, and 512±20 Ma[1-7] point to a Late Precambrian–Early Palaeozoic tectonomagmatic event. Among these granites, the Munnar granite, occurring in the high hills of the Western Ghats in the eastern part of Central Kerala (Figure 1), is the subject of the study.

Field relations and petrography

In the study area around Munnar, granite, gneissic granite, sheared gneiss, migmatite and calc-silicate rock are the chief rock types, traversed by minor quartz, pegmatite and aplite veins (Figure 2). The contacts of rocks are mostly not exposed due to thick vegetation and soil cover.

The general trend of migmatite and other rock units is WNW-ESE with dips of 35° to 65° towards north. Foliation trend shows local variations to WSW-ENE and occasionally to NNE-SSW. Local reversals of dip are also noticed (Figure 2). Megascopically, migmatite is a composite rock with alternating quartzo-feldspathic

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