# *Review of Literature*

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The concept of autoimmunity was first predicted by Nobel Laureate Paul Ehrlich at the start of the twentieth century, and he described it as 'horror autotoxicus'. His experiments led him to conclude that the immune system is normally timed to responding to foreign materials and has an inbuilt tendency to avoid attacking self tissues. But when this process goes awry, the immune system can attack self tissues resulting in autoimmune disease. The perplexing issue of what allows the immune system to attack self tissues is a continuing focus of research, as documented in the following review of literature.

The human immune system has the ability to discriminate self and non-self substances. Immunologic tolerance is the acquisition of immunosuppressive fervor and or non-responsiveness to self antigens, mainly through clonal deletion, clonal anergy and perhaps active suppressive mechanisms. If these self-tolerance mechanisms fail or breakdown, autoimmune disease results (Abbas et al., 2003).

## I. Immunological tolerance and autoimmunity

All individuals are tolerant to their own potentially antigenic substances, and failure of self tolerance is the fundamental cause of autoimmunity. The mechanisms of self tolerance have been worked out in considerable detail in animal models, and are best understood for $CD4^+$ T cells. Self tolerance can be divided into central tolerance and peripheral tolerance. In central tolerance, immature lymphocytes that happen to recognize self antigens in generative lymphoid organs (the bone marrow for B cells and the thymus for T cells) die by apoptosis; in peripheral tolerance, mature self-reactive lymphocytes encounter self antigens in peripheral tissues and are killed or shut off. The principal mechanisms of peripheral tolerance are anergy (functional unresponsiveness), deletion (apoptotic cell death), and suppression by regulatory T cells (Walker and Abbas, 2002). Autoimmune diseases develop when self-reactive lymphocytes escape from tolerance and are activated. Although the mechanisms by which this occurs are not entirely known, autoimmunity is thought to result from a combination of genetic variants, acquired environmental triggers such as infections, and stochastic events.

Our immune system is the body's sixth sense. It can react to any conceivable chemical structure to fight off every possible microorganism. The receptors coordinating this feat are antibodies expressed on the surface of B cells as B-cell receptors (BCRs) and T-cell receptors (TCRs) displayed on T cells. Huge receptor diversity is encoded in the mammalian genome by two processes of somatic genome modification that occur selectively in lymphocytes. First, V(D)J recombination assembles unique BCR and TCR genes from three separate gene segments, the variable (V), diversity (D) and joining (J) genes, during Band T-cell differentiation. This takes place in the 'central lymphoid tissues', which are principally the bone marrow for B cells and the thymus for T cells. Second, somatic hypermutation substitutes single nucleotides of BCR genes during a late phase of the immune response in peripheral lymphoid tissues (such as the spleen, lymph nodes and tonsils). A significant fraction of the receptors generated by both these processes bind to one or more self components in the body — a byproduct of a deliberately random receptor-generating process. Between 20 and 50 percent of TCRs and BCRs generated by V(D)J recombination bind with a potentially dangerous affinity to a self antigen (Ignatowicz et al., 1996; Laufer et al., 1996; Zerrahn et al., 1997; Wardemann et al., 2003). Since only 3–8 percent of the population develops an autoimmune disease (Jacobson et al., 1997), it is remarkable that this enormous burden of self-reactive receptors is so well regulated in most of us.

Each lymphocyte usually produces only a single receptor out of the billions possible. Experiments have established that if this receptor is self reactive, then four cellular strategies are employed to deal with them (Figure 1). First, the cell displaying the 'forbidden', or self-reactive, receptor can be triggered to die, as originally envisaged in Burnet's concept of clonal deletion. Second, a cell bearing a forbidden receptor can 'edit' the offending receptor by further V(D)J recombination or somatic hypermutation to display a different receptor that is not self reactive (Nemazee and Hogquist, 2003). Third, intrinsic biochemical and gene-expression changes can reduce the ability of the cell to be triggered by self-reactive receptors. This is generally termed clonal anergy or tuning (Healy and Goodnow, 1998; Grossman and Paul, 2000; Schwartz, 2003). Clonal anergy is a state of antigen unresponsiveness of a

clone of self-reactive immune cells, resulting in absence of cell mediated immune reaction in supposedly sensitized individuals. Therefore, T cell anergy may be defined as a tolerance mechanism in which the lymphocyte is intrinsically inactivated functionally following an antigen encounter, but remains alive for an extended period of time in a hyporesponsive state. Finally, even if the cells have evaded the three mechanisms above, collectively called 'immunological ignorance', extrinsic controls can limit the danger of self-reactive receptors. These extrinsic controls limit the supply of essential growth factors, costimuli, pro-inflammatory mediators and other factors, and also include active suppression by regulatory T (Treg) cells, through a mechanism that is poorly understood. These four mechanisms act as checkpoints on the pathway leading to the production of secreted antibodies and effector T cells.
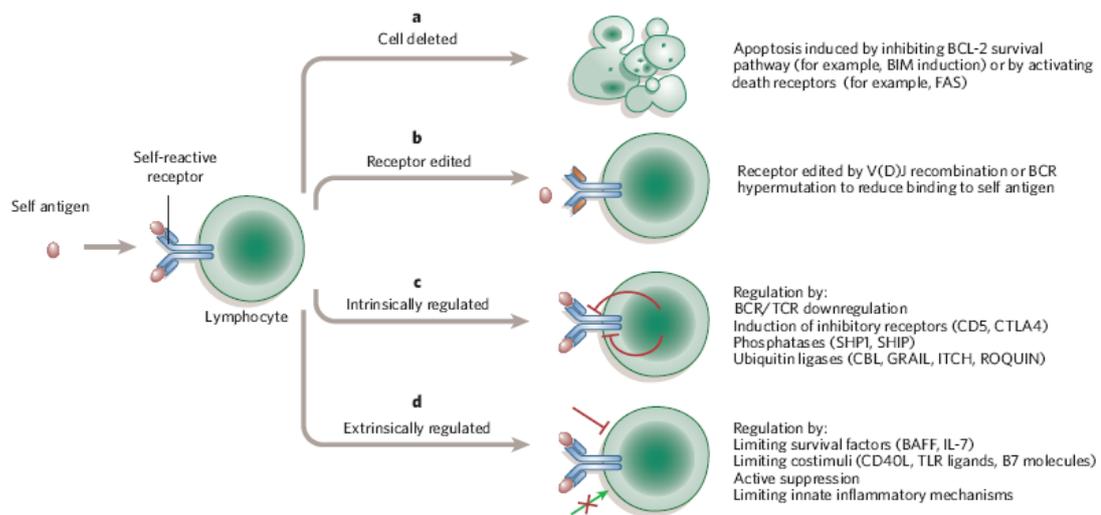


*[Goodnow et al., Nature 2005]*

**Figure 1:** Regulation of self-reactive receptors by four cellular strategies at different points during B- and T-cell differentiation.

## II. Genetic basis of autoimmunity

Genome-wide linkage studies attempt to identify genetic markers (and thus a genomic region) where there is more sharing of alleles between individuals (with a given trait) within families than is statistically expected. Such studies are most commonly used in identifying causal genetic variants for single-gene disorders. Genetic studies of simple traits undoubtedly met with early success than those of complex traits. However, recent advances in the study of complex traits are now contributing further to this

knowledge. In some cases, the genes identified in simple disorders will have more subtle alterations that confer susceptibility to a 'common' disease (that is, diseases caused by a combination of alleles); in other cases, the mutations will simply suggest which biological pathways may be implicated in common diseases.

Over the past decade, in autoimmunity as in many other human diseases, there has been great interest in testing candidate genomic regions (identified by linkage studies), or candidate genes (selected on the basis of their location under a linkage peak, or their known functional properties, or both) for evidence of their association with disease. Epidemiologic studies have demonstrated that genetic factors are crucial determinants of susceptibility to autoimmune diseases. There is familial clustering, and the rate of concordance for autoimmune disease is higher in monozygotic twins than in dizygotic twins (Gregersen, 1997; Ortonne, 1999; Kukreja and Maclaren, 1999). A few autoimmune diseases, such as autoimmune lymphoproliferative syndrome and the syndrome of autoimmune polyglandular endocrinopathy with candidiasis and ectodermal dysplasia (APECED), are due to mutations in a single gene. Even in these conditions, other genes modify the severity of disease and not all possessing the mutant gene manifest the disease. Autoimmune lymphoproliferative syndrome is an autosomal dominant disorder involving a defect in the fatty acid synthetase (Fas) protein or its receptor. The Fas pathway mediates apoptosis, which down-regulates immune responses (Drappa et al., 1996).

## A. Major histocompatibility complex (MHC)

Most autoimmune diseases are multigenic, with multiple susceptibility genes working in concert to produce the abnormal phenotype. In general, the polymorphisms also occur in normal people and are compatible with normal immune function. Only when present with other susceptibility genes do they contribute to autoimmunity (Becker, 1999; Encinas and Kuchroo, 2000). Some of these genes confer a much higher level of risk than others; for example, the MHC also called human leukocyte antigen (HLA) makes an important contribution to disease susceptibility. Most autoimmune diseases are linked to a particular class I or class II MHC molecule (Klein and Sato, 2000), but

this association may require linkage with another gene such as the one encoding tumor necrosis factor α (TNF-α) or complement.

A critical role in enhancing the autoimmunity risk is played by MHC haplotypes. MHC is a complex of genes with a high degree of polymorphism whose products are expressed on the surface of several cellular types. The MHC system is located on chromosome 6, and the whole allelic set on each chromosome is indicated as MHC haplotypes. It is possible that alleles from different loci are inherited in association (linkage disequilibrium). Similar haplotypes are often correlated with autoimmune diseases (Ohashi, 2003; Raman and Mohan, 2003; Larsen and Alper, 2004).

T lymphocytes are able to recognize specific antigens only when they are associated with MHC molecules on the cellular surface. Polymorphisms are fundamental to diversify the structure of MHC molecules and consequently allow the interaction with different processed antigens. Polymorphisms are also present in the binding site with T lymphocytes. This may condition a diverse binding affinity and increase the antigen presentation in the periphery, with a consequent enhanced T cell stimulation (Shlomchik et al., 2004). The T cell's capacity to interact with MHC molecules is also critical for their positive selection. Indeed, in the thymus, the $CD4^+CD8^+$ (double-positive) thymocytes, derived from bone marrow hemopoietic precursors, interact with cortical epithelial cells, which express a high level of MHC class I and class II molecules associated with self-peptides. Double positive cells bearing the TCR with appropriate affinity for peptide-MHC complexes are positively selected, thus ensuring that all mature T cells have some affinity for MHC molecules (MHC restriction). Thymocytes expressing TCRs that interact with peptide-MHC class I complexes become $CD8^+$ T cells, while those that express TCRs binding peptide-MHC class II complexes become $CD4^+$ T cells. However, peripheral double positive T cells have been demonstrated in several pathological conditions and in normal individuals (Germain, 2002; Parel and Chizzolini, 2004).

## B. Cytotoxic T lymphocyte antigen 4 (CTLA4)

CTLA4 (also known as CD152) is an inhibitory receptor expressed by T cells that recognizes the co-stimulatory molecules CD80 and CD86, the ligation of which shuts

off T-cell responses and promotes long-lived anergy (Salomon and Bluestone, 2001). CTLA4 works by competitively blocking the engagement of the activating receptor CD28 (by CD80 or CD86), and by transducing inhibitory signals; the latter probably involves tyrosine and serine/threonine phosphatase activation (Baroja et al., 2002). A striking association of *CTLA4* polymorphism with Graves' disease, type 1 diabetes and other endocrinopathies has been observed in knockout mice, resulting in reduced production of a truncated splice variant which has inhibitory activity (Ueda et al., 2003). The functional consequences of producing this altered form of CTLA4 have not been defined, but as expected for a complex disease, the biological effect of the causal allele is more subtle than what is found in monogenic disorders or in knockout mice. The findings of genetic studies in humans are consistent with these ideas. There are, for example, allelic variants of the CTLA-4 gene. One such polymorphism causes a small decrease in the inhibitory signal mediated by CTLA-4 and is associated with type 1 diabetes, thyroid disease, and primary biliary cirrhosis (Awata et al., 1998; Kouki et al., 2000; Agarwal et al., 2000). More often, however, a genetic locus rather than a single gene has been linked to susceptibility to autoimmune disease, and many loci are emerging as potentially important in more than one disease (Becker, 1999; Encinas and Kuchroo, 2000). The clinical observation that different autoimmune diseases often coexist within a family strongly suggests that some genes at these loci predispose patients to more than one disease (Ginn et al., 1998; Henderson et al., 2000).

## C. Autoimmune regulator (AIRE)

AIRE was identified as the gene that is mutated in autoimmune polyendocrine syndrome (APS-1) — a disorder that manifests as autoimmune attack against multiple endocrine organs, the skin and other tissues (Bjorses et al., 1998). In a study of functional genomics, the mouse homologue of the gene has been knocked out, and the AIRE protein shown to be responsible for the thymic expression of some antigens that are expressed at high levels in different peripheral tissues. In the absence of thymic expression, T cells specific for these antigens escape negative selection (central tolerance), enter the periphery and attack the target tissues (Anderson et al., 2002;

Liston et al., 2003). It remains to be determined whether defects in central tolerance contribute significantly to most multigenic autoimmune diseases (Mathis and Benoist, 2004).

## D. FoxP3 (encoding a transcription factor of the forkhead family)

FoxP3 (forkhead box P3) is a striking example of a gene whose role in autoimmunity has been revealed by the confluence of animal studies and studies of a quite rare human disease. CD4$^+$CD25$^+$ regulatory T cells, now established as major controllers of immune responses to self and other antigens (Sakaguchi, 2004), were shown to express high levels of FoxP3. Three groups demonstrated that induced knockout or spontaneous mutation of the mouse Foxp3 gene ('scurfy' mice) led to a systemic autoimmune disease associated with the absence of CD4$^+$CD25$^+$ regulatory T cells (Hori et al., 2003; Fontenot et al., 2003; Khattri et al., 2003). At the same time, a human disease known by the acronym IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) was shown to be associated with mutations in FoxP3. These results indicate that the development and/or function of regulatory T cells are dependent on the activity of this one transcription factor; its downstream targets and major functions are not yet clearly defined.

## E. Genetics of common autoimmune diseases

Genetic engineering of mice has led to the identification of at least 25 genes that can contribute to an autoimmune diathesis when they are deleted or over expressed. These genes encode cytokines, antigen co-receptors, members of cytokine- or antigen signaling cascades, co-stimulatory molecules, molecules involved in pathways that promote apoptosis and those that inhibit it, and molecules that clear antigen or antigen–antibody complexes. Two critical lessons have been learnt from these models. First, whether a particular gene or mutation causes a disease depends on the overall genetic background of the host: both disease susceptibility and the disease phenotype that result from an alteration of a single gene depend on other genes. Second, some genetic defects can predispose patients to more than one autoimmune disease, so that several diseases may share common pathogenic pathways. This

observation suggests the possibility of using common therapeutic strategies in different autoimmune diseases.

In contrast to simple diseases, common diseases are believed to result from a combination of susceptibility alleles at multiple loci, environmental factors (such as xenobiotics, pathogen exposure and hormone levels), and stochastic events. There is some debate as to whether common diseases are caused by common alleles of low penetrance (that is, alleles that confer modest increased risk to disease) or by multiple rare alleles of high penetrance. Although most disease alleles identified for common autoimmune diseases have the former characteristics, these may not represent the full spectrum of disease alleles. Our ability to discover as yet unidentified disease alleles has been improved by the recent explosion of knowledge regarding the common genetic variation in the human genome. Specifically, recent work has demonstrated that the vast majority of genetic variation in the human genome consists of individual bases that exist as either of two alleles in the population — known as single nucleotide polymorphisms (SNPs) — rather than as deletions or rearrangements. Approximately ten million SNPs in the human genome have a minor allele frequency greater than 1 percent and represent about 90 percent of the genetic variation in the human genome. Initial efforts to discover and map SNPs to the reference sequence of the human genome have resulted in a public resource containing most of these common SNPs (Wang et al., 1998; Sachidanandam et al., 2001; International Human Genome Sequencing Consortium, 2004).

Over the past decade, in autoimmunity as in many other human diseases, there has been great interest in testing candidate genomic regions (identified by linkage studies), or candidate genes (selected on the basis of their location under a linkage peak, or their known functional properties, or both) for evidence of their association with disease. Before the creation of public SNP databases, sequencing was needed to identify the genetic variants to test in an association study. Now it seems that there is almost an excess of SNPs to type. Therefore, it is important to understand the relationships that exist between these SNPs. First, by examining a high density of specific areas of the genome (Daly et al., 2001; Jeffreys et al., 2001; Patil et al., 2001), and by performing genome-wide surveys (Gabriel et al., 2002), it has become

clear that the alleles of the SNPs form patterns (also known as haplotypes) in the genome. Specifically, the emerging data demonstrate that alleles at nearby SNPs are highly correlated with one another (this is known as linkage disequilibrium). The tight correlation between SNPs, however, is broken down by recombination events resulting in haplotype blocks. Furthermore, the existing data and models based on these data suggest that, rather than occurring randomly, there are recombination hotspots in the genome (Jeffreys et al., 2001; Reich et al., 2002; McVean et al., 2004). Knowledge of the haplotype structure not only allows an optimal subset of SNPs to be selected that efficiently extracts the information about the common patterns of variation, but it also can direct how such data should be analyzed.

### a. Cytokine polymorphism in autoimmune diseases

Cytokines participate in the induction and effector phases of the immune and inflammatory response and are therefore likely to play a critical role in the development of autoimmune diseases. The cytokine network is complex, with cytokines having both diverse and overlapping functions, including effects that are promoted or inhibited by other cytokines. Cytokine secretion profiles can be considered as either pro- or anti-inflammatory or, alternatively, on the basis of the animal model as either T helper cell type 1 (Th1) responses promoting cell-mediated immunity [Interleukin (IL)-2 and interferon (IFN)-γ] or T helper cell type 2 (Th2) responses promoting humoral immunity (IL-4, IL-5, IL-6 IL-10, and IL-13) (Mosmann et al., 1989). Despite attempts to classify some autoimmune diseases as a classical Th1- or Th2-mediated disease, no clear conclusions can be drawn (Ajjan et al., 1996).

Gene polymorphisms that affect the function or the level of expression of regulatory or effector molecules of inflammation, fibrosis, or other pathological processes involved in autoimmune disease development have been observed; for example, in systemic sclerosis (SSc) transforming growth factors (TGFβ1, β2, β3), juvenile idiopathic arthritis (IL-1β), rheumatoid arthritis (RA; IL-4), systemic lupus erythematosus (SLE; IL-10), Sjogren syndrome (IL-10), juvenile idiopathic inflammatory myopathies [IL-1 receptor antagonist (RA)], and Wegener

granulomatosis (IL-10) (McDowell et al., 1995; Cantagrel et al., 1999; D'Alfonso et al., 2000; Rider et al., 2000; Susol et al., 2000; Anaya et al., 2002; Crilly et al., 2002; Zhou et al., 2002). In many autoimmune diseases (e.g. SLE, RA, Behcet disease, coeliac disease, inflammatory bowel diseases, and diabetes mellitus type 1), various TNF-α promoter or microsatellite polymorphisms have been found. The tumour necrosis factor polymorphism may be part of a disease-associated MHC haplotype (due to linkage disequilibrium) and represents a haplotype-dependent association (Hajeer et al., 1996; Sturfelt et al., 1996). Nevertheless, tumour necrosis factor polymorphisms as independent susceptibility factors for RA and SLE have been described in some populations (Martinez et al., 2000; Rood et al., 2000; Parks et al., 2004). Such polymorphisms may be directly involved in pathogenesis, because TNF-α is known to be the strongest inflammatory factor and a major candidate for long lasting immune response modifications, and associations of in vitro TNF-α production with tumour necrosis factor microsatellite polymorphisms have been reported (Pociot et al., 1993).

IL-1α and IL-1β are pleiotropic cytokines with primarily proinflammatory effects, including stimulation of IL-2 and IL-6. Both IL-1α and IL-1β as well as the naturally occurring IL-1RA act via the IL-1 receptor 1. Hence, disruption of the balance among IL-1α, IL-1β, IL-1RA, and their receptors may result in disease. Polymorphisms of IL-1α  (position -889) and IL-1β (exon 5, +3962) (Pociot et al., 1992) have been associated with juvenile RA (McDowell et al., 1995; Ploski et al., 1995) and insulin-dependent diabetes mellitus (Pociot et al., 1992; Pociot et al., 1994), respectively. Variation in the IL-1RA gene (exon 2) has been associated with a variety of autoimmune disorders (Blakemore et al., 1994; Mandrup et al., 1994; Mansfield et al., 1994; Blakemore et al., 1995; Blakemore et al., 1996). IL-4 mediates the humoral immune response, and polymorphisms in IL-4 (position -590) and its receptor gene (nucleotide 1902) have been associated with atopy (Rosenwasser et al., 1995; Hersey et al., 1997). IL-6 is a key inflammatory cytokine, and elevated systemic levels are seen in many conditions (Ajjan et al., 1996). Promotor region variation of the IL-6 gene has been associated with juvenile RA (Fishman et al., 1998). IL-10 enhances B cell proliferation and an IL-10 polymorphism (position -1082) has been associated

with SLE (Lazarus et al., 1997) and RA (Hajeer et al., 1998). TGF-β is another cytokine with important modulatory functions, including an inhibitory role in B cell maturation. Polymorphisms in genes encoding these crucial immunomodulatory molecules may result in an altered level of expression and hence must be considered important candidate genes for autoimmune disease susceptibility and severity.

Genetic factors likely play an important role in susceptibility to pemphigus, and its occurrence is significantly associated with several HLA class II alleles. From among them, HLA-DRB1*0402 and HLA-DQB1*0503 were identified as the strongest susceptibility alleles in different ethnic groups (Lee et al., 2006). Several studies have found increased concentrations and expression of cytokines IL-1α, IL-1β, TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8 and IL-10 in sera, blister fluids or affected skin of pemphigus vulgaris (PV) patients and described their correlation with disease activity, suggesting their role in disease pathogenesis (D'Auria et al., 1997; Feliciani et al., 2000; Bhol et al., 2000, 2001; Caproni et al., 2001; Loˊ pez-Robles et al., 2001; Keskin et al., 2008; Narbutt et al., 2008). TNF-α and IL-10 gene polymorphisms has shown an association with PV (Javor et al., 2010). The production or function of cytokines is, at least partially, regulated by polymorphisms in their gene sequences (Bidwell et al., 1999). Numerous cytokine polymorphic variants are associated with the development of a broad spectrum of autoimmune diseases, including skin-affecting disorders such as psoriasis vulgaris or SLE (Bidwell et al., 1999; Hollegaard and Bidwell, 2006). Thus, it is reasonable to hypothesize that certain cytokine polymorphisms also participate in genetic susceptibility to PV. So far, studies on their association with PV have been very scarce, probably due to low incidence of the disease throughout the populations.

**b. Genetic polymorphisms in xenobiotic-metabolizing enzymes**

Genetic polymorphisms of xenobiotic-metabolizing enzymes may result in expression of inactive enzymes or enzymes with a reduced or increased metabolic activity (Daly, 1995). For example, the incidence of hydralazine- and procainamide-induced lupus is higher or the disease starts earlier in individuals with the slow acetylator phenotype caused by mutant N-acetyl-transferase 2 (NAT-2) alleles than in individuals

exhibiting the fast-acetylator phenotype (Woosley et al., 1978; von Schmiedeberg et al., 1999).

The generation of covalent protein adducts formed after exposure to xenobiotics and neoantigens is another mechanism of drug-induced autoimmunity that is modulated by the polymorphism of metabolizing enzymes. Patients with dihydralazine- induced hepatitis are more often of the slow-acetylator phenotype (Siegmund et al., 1985). They produce autoantibodies against cytochrome P4501A2 (CYP1A2) due to a higher risk of adduct formation, because slow-acetylators can use only the pathway mediated by CYP1A2 for detoxification of dihydralazine. Polymorphisms of genes encoding S-mephenytoin 4-hydroxylases (CYP2C19) or organic solvent-metabolizing enzymes (CYP2E1) are associated with SSc. The CYP2E1*3 allele seems to be involved in increased susceptibility to scleroderma among individuals who have been exposed to organic solvents (Povey et al., 2001).

In conclusion, associations with genetic polymorphisms of xenobiotic-metabolizing enzymes would indirectly point to xenobiotics as etiological agents of immune-mediated diseases and may provide information as to the type of chemical compound to be searched for (Griem et al., 1998). A 3-fold increased risk of SLE associated with 24 or more months of occupational sun exposure with GSTM1 null genotype was observed, although the authors did not find any association of glutathione-S-transferase with an increased risk of lupus, nor did they find any association of occupational sun exposure with an increased risk of lupus (Fraser et al., 2003).

## III. Environmental triggers in autoimmune diseases

### A. Pesticides and xenobiotics

Xenobiotic compounds such as pesticides, are ubiquitous and nowadays an inescapable part of our environment (food, air, water, breast milk). They are found accumulated in the tissues of every person. The bioaccumulation of these compounds can lead to a variety of metabolic or systemic dysfunctions. The systems studied and found prominently affected are the immune, endocrine and neurological systems (Petri et al., 1997).

Pesticides are widely used worldwide in agriculture, public health, and several indoor conditions. There is some evidence, either in animal models or following human

exposure, that several pesticides used currently or in the recent past can cause slight changes that could be interpreted as "autoimmune-like effects". However, data supporting this hypothesis are scarce. Only few compounds that are no longer in use today (i.e. mercury derivatives, hexachlorobenzene) have been shown to induce more marked changes, suggesting their potential for causing autoimmune disease.

The interpretation of human data is difficult, not only because only slight and subclinical effects were observed, but also because human subjects usually are exposed to a mixture of several pesticides, thus making the identification of the role of a single ingredient very difficult. Some studies examined antinuclear or other autoantibodies in subjects exposed to pesticides in occupational settings. It is difficult to interpret studies using these measures if an appropriate comparison group is not included, given the prevalence of autoantibodies that has been reported in studies of healthy, unexposed subjects (Tan et al., 1997). Another major problem in data interpretation is the uncertainty in the extrapolation of animal findings to humans. One such example is the interpretation of enhanced antibody response to sheep red blood cells (Burns et al., 2000).

In humans, some epidemiological studies have reported increased levels of autoantibodies following exposure to pesticides. These include hormone supplementation, pesticides, insecticides, fungicides, and foods and herbal products. Epidemiologic studies suggest a possible link between pesticides and a variety of autoimmune diseases, but such an association remains speculative. Investigations of exposure to pesticides and estrogenic compounds have been areas of considerable research interest during the last decade. Organochlorine pesticides (OCPs) such as hexachlorocyclohexane (HCH), dichloro-diphenyl-trichloroethane (DDT), endosulfan, etc. differ from other chemical substances because these are purposively spread into the environment. As a consequence, a great part of the human population is exposed. Due to their persistent and lipophilic nature, these halogenated aromatic compounds bioaccumulate in the food chain and may contribute to acute and chronic illnesses. They can stimulate, suppress, or deregulate the immune system, depending on concentration and duration of dose, toxicity/bioreactivity, total body load, and the nutritional status of the individual. OCP that are known to alter the immune system

are DDT, chlordane, aldrin, lindane, hexachlorobenzene, mirex, and arochlor (Glick, 1974; Street and Sharma, 1975; Giurgea et al., 1978; Silkworth and Loose, 1980). Direct T-lymphocyte triggering is found with many OCPs. They often cause suppression of T-suppressor lymphocytes (Rea et al., 1986).

Certain major pesticide classes have been reported to have profound toxic effect on the immune system. DDT has been found to have effects on polymorphonuclear leucocytes, phagocytic activity, immune regulation, increased degranulation of mast cells, etc (Cullen, 1987). Pesticides may be immunomodulatory and, temporal association with a sensitizing exposure may demonstrate aggravation of, or precipitation of, autoimmune disease. Increased serum DDE (a metabolite of DDT) concentration was associated with increased total numbers of lymphocytes and decreased lymphocyte responsiveness to mitogen (Vine et al., 2001).

More recently, the prevalence of anti-nuclear antibody (ANA) in human sera was found to be associated with lifetime exposure to some pesticides, suggesting that occupational exposures to pesticides may be related to the development of SLE (Vine et al., 2001; Holsapple et al., 2002). Trichloroethylene (TCE) metabolites have been associated with SLE, SSc, and other autoimmune disorders. Similarly, a few epidemiologic studies have examined occupational exposures to dioxins (Gilbert et al., 1999).

The past decades have seen tremendous progress in biomolecular research on the autoimmune process in pemphigus, and most of the involved antigens have been traced. There are, however, no equivocal conclusions regarding the etiology of the disease. The prevailing concept is that pemphigus stems from a combination of endogenous and exogenous factors in genetically susceptible individuals (Brenner and Ruocco, 1994; Bronson et al., 1997). The exogenic factors are numerous and include drugs (Brenner et al., 1997), ultraviolet (UV) radiation (Kerker and Morison, 1990), stress (Plumb and Doolittle, 1996), infection (Grunwald et al., 1986; Brenner et al., 1997), hormones (Ruach et al., 1995), and others (Kerker and Morison, 1990; Bellaney and Rycroft, 1996).

A study has indicated a correlation between occupational exposure of pesticides to patients living in rural settings and pemphigus (Wohl and Brenner, 2003). Exposure to

pesticides in rural areas is greater than in the urban areas. Krain, 1974 reported the first series of 14 cases that developed pemphigus following occupational contact with various chemical substances, gardening materials and pesticides being the most incriminated. Since that report, case studies of pemphigus induction by phosphamides, chrome salts (Tzankov et al., 1990), pentachlorophenol (Lambert et al., 1986) and other agents continue to appear in the literature.

There are several mechanisms that could explain how pesticides are involved in pemphigus pathogenesis. Many of them can cause a direct toxic effect to desmosomes, while others activate the immune system, specifically the Th2 arm with its array of cytokines, generating autoantibodies (Robinson et al., 1999). A recent hypothesis holds the acetylcholine receptor, a surface protein and a molecule altered by different pesticides, as also being a target of pemphigus antibodies (Grando, 2000). Chronic exposure to metals can induce autoantibodies and immune complex mediated glomerulonephritis (Hueser, 1992). Organophosphate/ organochlorine pesticides have an enhancing effect on autoimmune disorders and may also aggravate the clinical state by jeopardizing the immunosuppressed or anergic state of such individuals (Lindqvist et al., 1974), depending upon which type of cells in an autoimmune disease patient bears the brunt of the exposure. Despite large amounts of experimental data (Vial et al, 1996) suggestive of pesticide induced immunosuppression, definite proof of association, causality between autoimmune disease and evidence of pesticide exposure is still lacking.

## B. Infectious agents

Disparate social and environmental factors and medical status have been associated with the onset of autoimmune disorders. Infectious agents may induce the breakdown of immunological tolerance and the appearance of auto-reactivity. There is evidence that the development of certain autoimmune diseases may be associated with a bacterial or viral infection that stimulates production of antibodies and immune cells called T cells, which are targeted against bacterial proteins that closely resemble "self" proteins, leading to cross reactivity with healthy tissues. The auto-reactive T cells may play a useful role in promoting the immune response to infection. However,

the specific relationship between infection and autoimmunity is still unclear. The exact mechanisms by which infection induces a particular autoimmune disease are unknown. In the case of streptococcus, it is believed that an antigen of the microorganism resembles an antigen present in the heart and that a cross-reactive immune response to the infecting microorganism causes immune-mediated damage to the heart. The phenomenon is referred to as molecular mimicry between the infectious agent and self. The concept of molecular mimicry is a viable hypothesis in the investigation of the etiology, pathogenesis, treatment, and prevention of autoimmune disorders. In other instances, microorganisms or local inflammation may alter antigens of the host so that the immune system sees them as foreign. Infections may also increase immune cell expression of co-stimulatory molecules and thus promote autoimmune responses.

Microbial superantigens (sAgs) are known to cause toxic shock, and are reasonable candidates for a role in induction of autoimmunity. A classic example is the central role of the group A beta-hemolytic Streptococcus in the development of rheumatic heart disease. Acute Guillain-Barré syndrome, an acute autoimmune demyelinating polyneuropathy, has been associated with a number of bacterial and viral infections, and reactive arthritis has been linked to a variety of intestinal infections (viral or *Campylobacter jejuni* infections). Indirect evidence has implicated a number of infections in type 1 diabetes and multiple sclerosis, and it has focused renewed attention on the possible role of Epstein-Barr virus (EBV) in SLE and RA. Thus, differences between the stimulation of male and female innate immune responses by pathogens must also be studied (Fairweather et al., 2001; Guarneri et al., 2005).

Candidiasis, either mucocutaneous or systemic, predominantly caused by the commensal *Candida albicans* can be seen in healthy individuals but more often affect immunocompromized individuals (Yu et al., 2007). Cell-mediated immunity plays an important role in defending and clearing of this pathogen. It has been shown that CD4$^+$ T cells and the p40 subunit of IL-12 and IL-23 are strict prerequisites for resistance. Resistance to this yeast is found to be associated with Th1 immunity while Th2 immunity is associated with susceptibility to systemic infection (Saunus et al., 2008). Natural killer cells and CD8$^+$ cytotoxic T cells are involved in damaging and

clearing this pathogen (Palma-Carlos et al., 2002). The discovery of autoimmunity to certain key defenses against Candida has helped to unravel the mystery of the chronic mucocutaneous candidiasis seen regularly or occasionally in the autoimmune syndromes in APECED or thymoma patients, and also its mucocutaneous restriction. Recently, evidence has been provided that IL-22 and possibly IL-17F are crucial in protection against mucocutaneous Candida infection (Kisand et al., 2011).These findings have even broader clinical and pathogenetic implications. There is a provocative evidence for identification of a *Candida albicans* protein responsible for superantigen-like effects which is derived from the observation that human peripheral blood mononuclear cells (PBMCs) incubated with *C. albicans* skin test antigen increased gene transcripts for Vβ subsets 5.1 and 5.2 (Walsh et al., 1996). The amino terminus of the *C. albicans* cell wall protein Int-l, named for its integrin-like sequence and putative roles in adhesion, morphogenesis, and virulence (Gale et al., 1998), displays 56 percent identity with the MHC class II binding site of the well-characterized superantigen MAM from *Mycoplasma arthritidis* (Cole et al., 1996).

In recent years, environmental agents such as bacterial sAgs have been demonstrated to contribute to the deregulation of immune responses, resulting in autoimmunity or immunodeficiency (Schiffenbauer et al., 1998). Superantigens are potent activators of CD4^{+} T cells; are capable of activating a large proportion of circulating T cells irrespective of their antigen specificity, thereby causing high levels of cytokine release into the circulation. It has been hypothesized that sAgs influence the course of autoimmune disorders, either by initiating (triggering) the immune process or by inducing a relapse in an individual who maybe in clinical remission. The possible ways that bacterial/viral sAgs trigger autoimmune mechanisms can either be by activation of auto-reactive T cells/ B cells or, activation of antigen presenting cells (APCs). Superantigens can directly activate auto-reactive T cells, which can then migrate to the appropriate site, take up residence and mediate autoimmune destruction through the release of cytokines or through cytotoxic mechanisms (Morshed et al., 2000). Activation of auto-reactive B cells can occur through MHC-II sites on B cells or through sAgs mediated activation of macrophages, releasing cytokines and causing inflammation.

In developing countries, helminthic infections/ infestations affect millions of individuals. The parasitic prevalence in Indian population is high, although the type of infestation varies in different regions. The complexity of the life cycle of helminthes (most commonly hookworm) offers numerous opportunities for the parasite and host to interact at the molecular level. Eosinophils play an important role in engaging the multicellular helminthic parasite. The binding of eosinophil to parasite is mediated by raised circulating immunoglobulin (Ig)G or IgE antibody deposited on the surfaces of the parasite. Eosinophilia via lipid mediators (leucotriene C1/platelet activating factor) can be toxic to the helminthes. The antibody response is in turn under the control of Th2 cytokines due to heavy parasitic infestation typically mediated by IL-4, promoting IgE synthesis and eosinophil production. Increased levels of IL-4, IL-5, IL-10 and reduced levels of IL2, IFN-γ determine the outcome of parasitic infection. The cytokine synthesis inhibitory factor (CSIF, IL-10) produced by activated Th2 inhibits the production of cytokine by Th1 and therefore indirectly enhance Th2 growth. These infestations too lead to persistent activation of immune system and induction of a hyporesponsive and/ or anergic state (Bentwich et al., 1995; Bentwich et al., 1999). Therefore, the capacity of highly parasitized individuals to mount protective immune responses becomes debatable and demands further study and elucidation.

The environment can also affect the immunoreactivity of the individual by shifting the balance of T cells within the individual between inflammatory, IFN-γ producing Th1 cells and IL-4-and IL-5-producing Th2 cells. Directly or indirectly, bacterial and viral infections usually induce T cell differentiation into Th1 cells. Infectious agents affect the ability of T cells to detect self antigens by cross-reaction (molecular mimicry). Many autoimmune diseases are much more common in women than in men. However, autoimmune diseases that develop in men often are more severe. The age at which the infection occurs may also influence the development of autoimmune disease (Kukreja et al., 2000; Fairweather et al., 2004).

**a. Bacterial superantigens**

The term "superantigen" was coined to describe the strong TCR variable (Vβ)-restricted primary response to bacterial toxins (Marrack and Kappler, 1990). This has

led to a flurry of work on the functional and structural properties of the new type of immunogen. A family of exotoxins [called superantigens (sAgs)] of the gram-positive cocci, *Staphylococcus aureus* and *Streptococcus pyogenes*, are among the most powerful T cell activators known (Proft and Fraser, 2003). Staphylococcal enterotoxin B (SEB) is one of the many potent pyrogenic exotoxins produced by *Staphylococcus aureus*, and a prototype bacterial superantigen (Dinges et al., 2000). SEB causes a variety of clinical diseases ranging from mild and often self-limiting food poisoning to lethal non-menstrual toxic shock (Dinges et al., 2000; Ferry et al., 2008). SEB is one of the major virulence factors in the context of staphylococcal infections caused by antibiotic-resistant strains (Fey et al., 2003), which represent a growing public health concern. Various preventive and therapeutic strategies have been tested in experimental animals to counter the toxicity and lethality of bacterial superantigens, including SEB. These include passive administration of neutralizing antibodies against SEB (Hamad et al., 1994), SEB toxoid vaccines (Boles et al., 2003), recombinant SEB mutants with attenuated immunotoxicity (Stiles et al., 2001; Coffman et al., 2002) and engineered high-affinity binding inhibitors (Buonpane et al., 2007; Yang et al., 2008). However, there are currently no effective treatments or vaccines approved for human use against SEB.

Deep tissue infections by *Streptococcus pyogenes* can also produce equally powerful sAgs capable of causing lethal shock (Kotb and Fraser, 2008). Interestingly, the sAg induced immune response is not targeted at the bacteria themselves, but rather sAgs function to direct a nonspecific T cell- and cytokine-mediated immune response that somehow assists in bacterial survival. Although many cytokines are produced in response to a single sAg, acute toxicity is blamed on the excessive production of three T cell cytokines— IL-2, IFN-$\gamma$, and particularly TNF-$\alpha$ (Miethke et al., 1993; Bette et al., 1993). Molecular mimicry between streptococcal M protein-5 and heart-tissue proteins, combined with high inflammatory cytokine and low IL-4 production, leads to the development of autoimmune reactions and cardiac tissue damage in rheumatic heart disease patients (Fae et al., 2005). Guilherme et al., 2007 identified several M protein epitopes recognized by peripheral T cells of rheumatic fever/ rheumatic heart disease patients and by heart tissue infiltrating T cell clones of severe rheumatic heart

disease patients. These T cells are CD4^{+} and produced inflammatory cytokines TNF-α and IFN-γ (Guilherme et al., 2010).

Superantigens bind directly to α- or β-chain of cell surface MHC class II molecules (outside of the peptide binding groove), without undergoing any intracellular processing. Subsequently, they activate T cells by interacting directly with the variable region of the β-chain (in rare cases α-chain) of the TCR, irrespective of their antigen specificities (Li et al., 1999). T cell activation by superantigens does not require CD4 or CD8 co-receptors. Thus, MHC class II-bound sAgs can vigorously activate both CD4^{+} and CD8^{+} T cells in a TCR dependent but cognate antigen-independent manner. Superantigens are thus able to activate a large pool of T cells (30–70 percent of total T cells) as opposed to the very low T cell frequency (1 in $10^4$ to 1 in $10^6$) for conventional peptide antigens.

Mutagenesis studies have mapped the binding sites for a number of different sAgs and co-crystal structures of sAgs bound to either MHC class II or TCR have confirmed their location (Jardetzky et al., 1994; Li et al., 2001). What has been most surprising is the variety of binding modes used by individual sAgs (Figure 2). For example Staphylococcal enterotoxins - SEB and SEC - cross-link MHC class II α-chain and TCR β-chain. Streptococcal pyrogenic exotoxin C (SPEC) binds to the other side of MHC class II and cross-links TCR β-chain while Staphylococcal Enterotoxin A (SEA) binds both α- and β-chain binding sites on MHC class II to cross-link TCR β-chain. Staphylococcal enterotoxin H (SEH) is the only sAg that binds to a TCR α-chain (Saline et al., 2010). Thus, although sAgs share a very similar protein structure, each has evolved its own way of binding to MHC and TCR—and this remarkable binding diversity clearly offers *S. aureus* and *S. pyogenes* an important survival advantage.

Bacterial superantigens robustly activate CD4^{+} as well as CD8^{+} T cells (Li et al., 1999). The CD4^{+} T cells could be classified into Th0, Th1, Th2, and Th17 cells based on their cytokine secretion profiles (Bettelli et al., 2007). The Th0 are naïve precursor cells or the uncommitted cells that primarily produce IL-2 upon activation. Th0 cells following activation can differentiate into Th1, Th2, or the Th17 pathways depending on the local cytokine milieu (Zhu and Paul, 2008). Th1 cell types mainly produce

IFN-γ; Th2 cells, IL-4, 5, and 13; and Th17 cells, IL-17, and IL-22. During a classical immune response, the differentiation of T helper subsets from Th0 to Th1, Th2, or T17 types occurs over days, if not weeks.

The CD8^{+} T cells can also be classified as cytotoxic T (Tc)1, Tc2 cells (Sad et al., 1995), and probably Tc17 cells (He et al., 2006), based on the cytokines they secrete. As bacterial sAgs can robustly activate CD8^{+} T cells expressing the appropriate TCR Vβ elements, the CD8^{+} T cells could be also contributing to the cytokine storm in response to these antigens. The other primary effector function of activated CD8^{+} T cells is cytotoxicity. CD8^{+} T cells mediate cytotoxicity primarily through granzyme B (Lord et al., 2003). Some reports have shown that CD4^{+} T helper cells can also contain granzyme B and mediate cytotoxicity. Irrespective of the source, granzyme B can cause death of the target cells by several mechanisms.
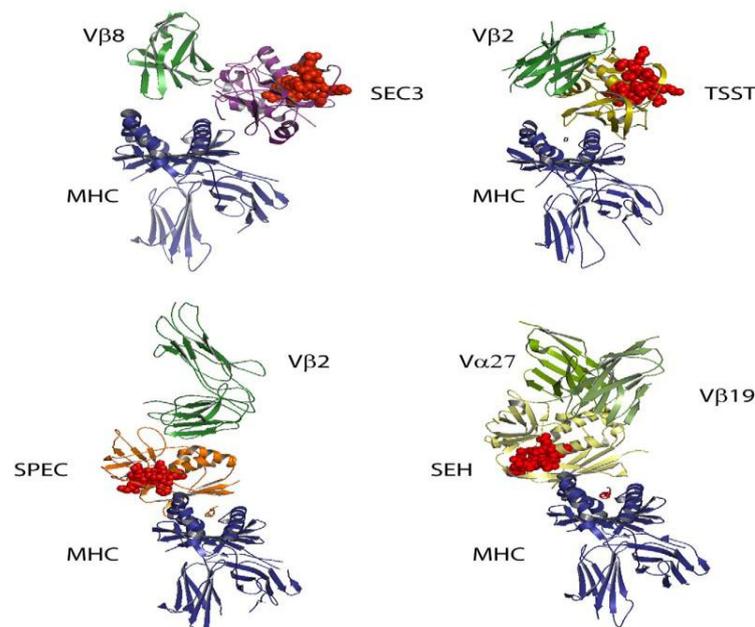


*[Fraser JD, PLoS 2011]*

**Figure 2:** The diversity in superantigen binding as shown by the model structures of four trimer complexes.

**b. Viral agents**

Viral infections are the main candidate environmental factors in the initiation and promotion of autoimmune diseases owing to their capacity to elicit strong immune activation and induce autoimmune diseases in animal models, as well as their correlation with autoimmune diseases in humans. There are five main mechanisms through which an infector can contribute to the pathogenesis of an autoimmune disease (Wucherpfennig, 2001). First is molecular mimicry, where the infecting agent may incorporate an epitope that is structurally similar to a self-antigen (Wucherpfennig, 2001). This structural resemblance confuses the immune system and induces the autoimmune response. Another mechanism is a phenomenon known as epitope spreading, where an exaggerated local activation of antigen presenting cells due to an inflammatory state may cause over processing and over presentation of antigens. In chronic autoimmune disease, this exaggerated activation may cause the priming of large numbers of T cells with broad specificities, thus encouraging the development of the autoimmune disease (Lehmann et al., 1992). Viral and bacterial sAgs possess the ability to bind to the variable domain of the T cell receptor beta chain along with the ability to bind to a wide variety of MHC class 2 molecules. These capabilities allow them to bind to a wide variety of T cells, irrespective of their specificity and to therefore induce an autoimmune reaction (Schereret al., 1993). Bystander activation is a mechanism describing a situation where enhanced cytokine production induces the expansion of autoreactive T cells whose prior number was insufficient to produce an overt disease (Murali-Krishna et al., 1998).

The last mechanism is known as polyclonal activation or activation of lymphocytes by lymphotrophic viruses. In this case, an infection of B cells results in B cell proliferation, enhanced antibody production, and the generation of circulating immune complexes that may cause damage to self-tissues (Ferri and Zignego, 2000). EBV and cytomegalovirus (CMV) are notorious for these associations.

 EBV was first associated with autoimmune diseases in 1971, when a high prevalence of the virus was found in the sera of SLE patients (Evans, 1971). Over the years, this relationship was strongly established, and today it is known that EBV infection preceding the development of autoimmune diseases may contribute to the

pathogenesis of SLE via the mechanism of molecular mimicry of its nuclear antigen 1 (EBV NA-1) and lupus-associated autoantibodies, such as anti-Smith antigen and anti-Ro (antibodies targeting protein antigens associated with small RNA molecules) (Poole et al., 2006; Harley et al., 2006). Aside from SLE, EBV infection is currently associated with multiple chronic autoimmune diseases (Pender, 2003), such as RA (Costenbader and Karlson, 2006), multiple sclerosis (Haahr and Hollsberg, 2006), Sjogren's syndrome (Trimeche et al., 2006), autoimmune thyroiditis (Vrbikova et al., 1996), and autoimmune hepatitis (Vento et al., 1995).
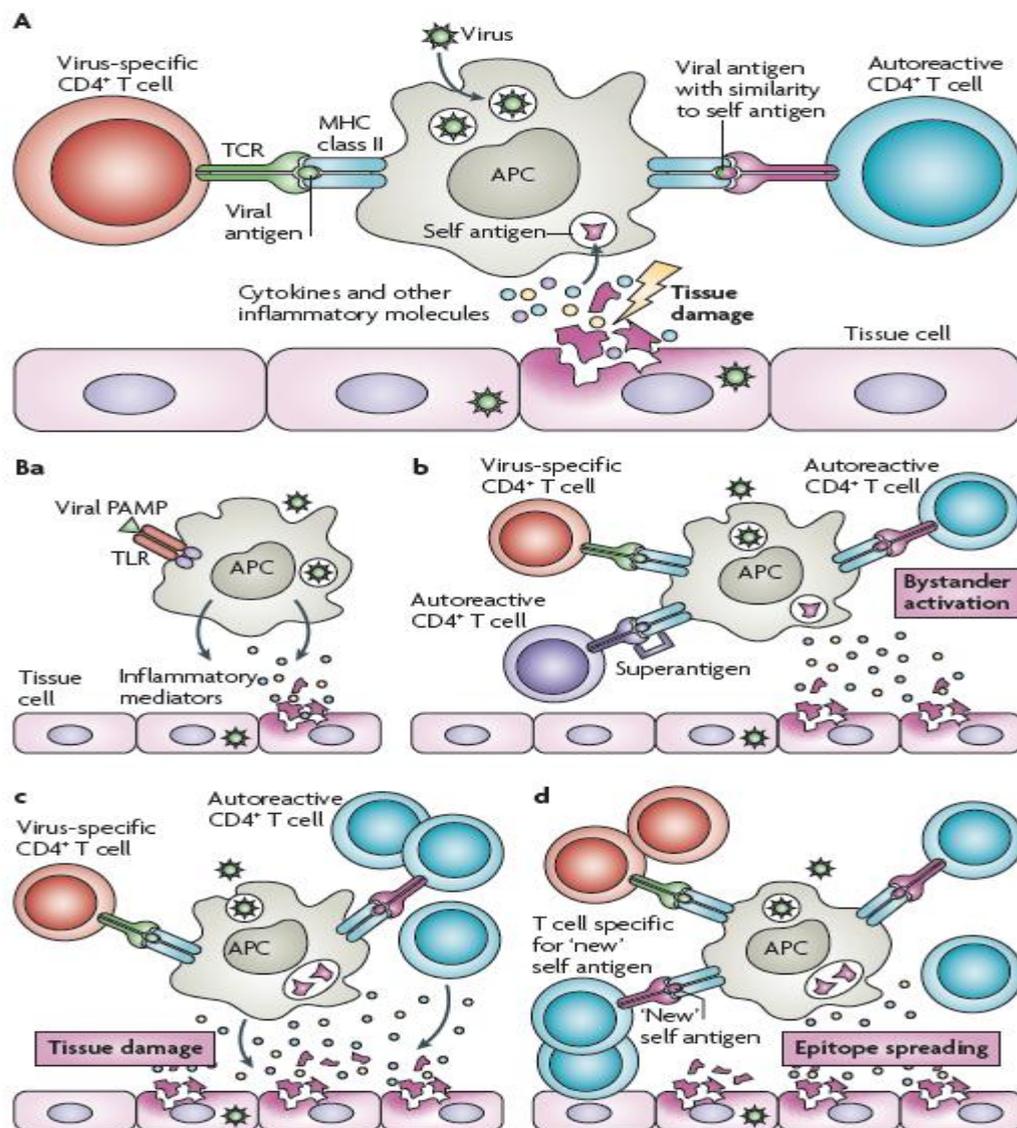


*[Munz et al., Nature Reviews Immunology 2009]*

**Figure 3:** Mechanisms of infection-induced autoimmunity.

CMV is another common viral infection of the herpes family associated with autoimmune diseases, including SLE (Sekigawa et al., 2002), diabetes mellitus type 1 and 2 (Filippi and Von Herrath, 2005; Roberts and Cech, 2005), inflammatory bowel diseases (Criscuoli et al., 2006), and SSc (Guiducci et al., 2004; Varani and Landini, 2011). A single report has associated CMV infection with antiphospholipid syndrome (Uthman et al., 1999). Finally, data associating CMV infection with atherosclerosis exist alongside with conflicting data denying this link (Nerheim et al., 2004). CMV IgG titers were found elevated in the sera of SLE patients, strengthening this known theory of association (Barzilai et al., 2007). On the other hand, patient sera from multiple autoimmune diseases were found to encompass elevated CMV IgM titers, perhaps pointing out that not all is known regarding the associations of CMV and autoimmune diseases.

A majority of SLE patients have antibodies to the C-terminus of CMV structural protein pp65, in particular to the sub-fragment $pp65_{336-439}$ (Hsieh et al., 2011). Immune reactivity against this peptide was also found in 14 to 20 percent of patients with RA, Sjögren's syndrome, and SSc but in only 4 percent of healthy controls. Immunization of BALB/c mice with $pp65_{336-439}$ and C3b adjuvant produces multiple antibodies that recognize nuclear structures, including chromatin, centriole mitotic spindle type I/II, and double-stranded DNA, and the immunized mice showed immunoglobulin deposition in kidney glomeruli (Hsieh et al., 2011). Previously, this group showed that pp65 immunization induces early autoantibody production and glomerulonephritis in lupus-prone mice. These observations suggest that the human CMV $pp65_{336-439}$ peptide elicits production of antibodies that cross-react with nuclear proteins and are pathogenic in genetically susceptible persons.

After a primary infection, CMV resides in latently infected monocytes or premonocytic cells, and reactivation often driven by inflammation may occur periodically (Varani and Landini, 2011). Even though the virus may not trigger autoimmune disease, it may be reactivated by an initial inflammatory insult and thereafter sustain and exacerbate inflammatory processes by producing type I cytokines and by specific mechanisms that induce inflammation and autoimmune reactions. For example, human CMV infection induces expression of cyclooxygenase-

2 and 5-lipoxygenase and induces production of prostaglandin E2, leukotriene B4, and IL-6 − all potent inflammatory mediators (Zhu et al., 2002; Qiu et al., 2008; Slinger et al., 2010) . Human CMV proteins alone will also drive immune reactions to nonself-peptides. By definition, autoimmune reactions occur in the absence of a pathogen in the affected organ. Emerging evidence, however, suggests that CMV proteins are common in tissues affected by autoimmunity and that both molecular mimicry and viral antigens may sustain immune reactions.

Active CMV infections are indeed frequent in patients with SLE, and the virus has been implicated in both development and progression of the disease (Varani and Landini, 2011). CMV RNA has been detected in endothelial cells in skin biopsies from patients with autoimmune sclerosis, and CMV infection is associated with higher disease activity scores in SLE patients (Varani and Landini, 2011). Poor immune control of CMV may lead to sustained periods of infection, and hyperfunctional Toll-like receptor (TLR) phenotypes may enhance the risk of autoimmunity. CMV may be an important trigger of autoimmunity in genetically susceptible persons, perhaps especially in patients with SLE who develop CMV-specific antibodies that recognize nuclear structures and double-stranded DNA, which are highly prevalent in these patients. Their hyperfunctional phenotype with regards to high IFN-α levels connects to TLR functions, which may explain the genetic susceptibility of CMV-induced pathologies in this patient group (Soderberg-Naucler, 2012).

## IV. T cells, T-cell receptor and autoimmunity

T-cells can be characterized by the structure of their antigen receptor, the TCR. Multiple gene segments are spliced and recombined in order to produce a TCR (Rowen et al., 1996; Oltz, 2001). The gene segments used to encode the TCR will determine its primary, secondary, and tertiary structure, and therefore the antigenic specificity of the T-cell (Matis, 1990; Schumacher, 2002; Kjer-Nielsen et al., 2002). Investigations of the gene segments used to encode the TCR of pathogenic T-cells are vital to our understanding of autoimmune disorders.

Immature double-positive T-cell precursors express both CD4 and CD8 co-receptors on their cell surface (Chan et al., 1994; Ellmeier et al., 1999; Magalhaes et al., 2006). As they mature, T-cells will develop into single-positive CD4$^+$ or CD8$^+$ T-cells by losing the expression of one of these co-receptors. The single non-antigen specific CD4 or CD8 co-receptor that is retained by the T-cell will determine the T-cell's potential effector function (O'Garra and Murphy, 1993; Zamoyska, 1998). T-cell effector function is induced when the T-cell encounters its specific antigen presented by APC (Larsson et al., 1984; Croft, 1994). APCs express cell-surface MHC molecules that belong to one of two classes. MHC class I molecules present peptides derived from intracellular pathogens found in the cytoplasm to CD8$^+$ T-cells (Teyton and Peterson, 1992; Yewdell et al., 1999; Williams et al., 2002). MHC class II molecules present peptides derived from extracellular and intravesicular pathogens to CD4$^+$ T-cells (Teyton and Peterson, 1992; Sant, 1994; Villadangos, 2001). In autoimmune disorders, autoreactive T-cells are activated by MHC presentation of self-peptides. Activated CD8$^+$ T-cells differentiate into cytotoxic T-cells that kill the peptide-presenting target cell (Larsson et al., 1984; Weninger et al., 2002) and activated CD4$^+$ T-cells differentiate into helper T-cells that activate or suppress other immune cells (Larsson et al., 1984; Ansel et al., 2003). The main groups of helper T-cells are Th1 cells, which release macrophage-activating cytokines such as IFN-γ and TFN-α (Munoz Fernandez et al., 1992; Billiau et al., 1998; Murphy and Reiner, 2002; Ansel et al., 2003), Th2 cells, which release B cell-activating cytokines such as IL-4 and IL-5 (Valle et al., 1989; Takatsu, 2004), and Th3 cells, which inhibit inflammatory T-cell responses by secreting the cytokines IL-10 and TGF-β (Weiner, 2001; Mills and McGuirk, 2004).

T-cells are able to recognize specific antigens complexed with MHC molecules on APC through the TCR (Zinkernagel and Doherty, 1974; Larche, 2008). The TCR is a heterodimeric cell surface receptor composed of an α-chain and a β-chain, which are transmembrane glycoproteins (Allison et al., 1982; Garcia et al., 1999). Each glycoprotein chain of the TCR is composed of a highly diverse amino-terminal variable region and a highly conserved carboxy-terminal constant region (Chan and Mak, 1989). The variable region of the TCR α-chain is encoded by a V and a J gene

segment (Sim et al., 1998). The TCRα locus is on chromosome 14 in humans and includes 70-80 Vα gene segments and 61 Jα gene segments (Boehm and Rabbitts, 1989). The variable region of the TCR β-chain is encoded by a V, a D, and a J gene segment (Chan and Mak, 1989; Rowen et al., 1996; Sim et al., 1998). The TCRβ locus is on chromosome 7 in humans and includes 52 Vβ gene segments, 2 Dβ gene segments, and 13 Jβ gene segments (Rowen et al., 1996; Sim et al., 1998). These gene segments get randomly spliced and recombined so that only one of each gene segment is used to encode each TCR (Shinkai et al., 1992; Rowen et al., 1996; Oltz, 2001). The number of variable gene pair combinations for αβ T-cells is roughly $5.8 \times 10^6$; however, due to junctional diversity between the gene segments where extra nucleotides may be added, the total diversity is about $10^{18}$ possibilities (Amzel et al., 1974; Sim et al., 1998). TCR Vβ gene segment usage has been shown to be restricted in some autoreactive T-cells and this skewing may be related to the development and course of autoimmune diseases (Sumida et al., 1997; Sutmuller et al., 1998; Heufelder, 1998).

Variability in each chain of the TCR resides in three hypervariable loops with less variable framework regions in between (Jorgensen et al., 1992; Johnson and Wu, 2000). The hypervariable loops are called complementarity determining regions CDR1, CDR2, and CDR3 and they are responsible for making direct contact with the antigen-MHC complex (Jorgensen et al., 1992) and determining T-cell antigenic specificity (Bentley and Mariuzza, 1996; Davis et al., 1998). CDR1 (roughly residues 30-36) and CDR2 (roughly residues 49-65) loops of the TCR are less variable and allow for intrinsic specificity for MHC molecules (Sim et al., 1998; Al-Lazikani et al., 2000; Johnson and Wu, 2000). CDR3 (roughly residues 95-103) is the most diverse hypervariable region due to somatic recombination, which is a process unique to lymphocytes and consists of splicing and recombining the germline DNA so that only one V, D (for the β-chain), and J gene segment are used to encode the TCR (Al-Lazikani et al., 2000). Further diversification of the CDR3 is provided by imprecise joining in which non-templated nucleotides can be added in between the V, (D), and J gene segments (Hughes et al., 2003). All of this diversity contributes to the vast T-cell repertoire of an individual because which gene segments combine together determines

the structure of the TCR, and therefore determines each T-cell's antigenic specificity (Matis, 1990; Kjer-Nielsen et al., 2002; Schumacher, 2002). Interestingly, conserved CDR3 amino acid motifs have been found in many autoimmune disorders supporting the notion that infiltrating T-cells recognize specific epitopes on autoantigens (Sumida et al., 1997).

The processes leading from autoimmunity to autoimmune disease are still in question. How autoreactive T-cells lead to tissue destruction in susceptible individuals but not in others is crucial to our understanding of the development of autoimmune diseases. It's possible that individuals with autoimmune diseases may be genetically prone to autoimmunity (Stefanova et al., 2002). If so, this susceptibility may be due to fine specificity differences of the responding autoreactive T-cells. Because TCR diversity is created by random gene segment rearrangements, the T-cells must go through positive and negative selection processes in the thymus to ensure that an individual develops a functional T-cell repertoire that is not strongly autoreactive. Both of these selection processes are dictated by the interaction of the TCR with the antigen:MHC complex and are dependent upon antigenic specificity (Marusic-Galesic and Pavelic, 1990; Larche, 2008). Positive selection of thymocytes is mediated by thymic cortical epithelial cells that express MHC molecules (Lo et al., 1997; Magalhaes et al., 2006). If the TCR is able to recognize a peptide:MHC complex with low affinity, the T-cell will receive an undefined survival signal and will be positively selected to mature (Marusic-Galesic and Pavelic, 1990; Stefanova et al., 2002). This positive selection process gives rise to a population of thymocytes that is restricted for self-peptides and self-MHC molecules. Negative selection of thymocytes is most efficiently mediated by bone marrow-derived dendritic cells and macrophages (Lo et al., 1997; Magalhaes et al., 2006). If the TCR is engaged by a peptide:MHC complex with high affinity, the immature T-cell will be negatively selected and die by apoptosis (Marusic-Galesic and Pavelic, 1990; Goodnow, 1996). Because the dendritic cells and macrophages are the same cells that activate mature T-cells in the peripheral lymphoid tissues, it is important to eliminate any self-reactive T-cells while they are still in the thymus so that they cannot react to self-antigens in the periphery. This negative selection process gives rise to a population of self-tolerant T-cells. Because of their role in positive and

negative selection, MHC molecules are essential in filtering out autoreactive T-cells and in shaping the T-cell repertoire that makes its way into the periphery. Characterization of the autoreactive T-cells that are chosen by MHC molecules to mature and make their way into the periphery will better our understanding of how autoimmunity progresses to an autoimmune disease in susceptible individuals.

## A. Regulation of immunity by self-reactive T cells

In the past, autoimmune diseases have been studied on the basis of the organ affected, but in recent years the focus has switched to a cross disciplinary approach with a view to providing a better understanding of the common mechanisms underlying the pathogenesis of these diseases. The immune system has evolved to recognize and combat infectious agents (Janeway, 1989). From a teleological point of view, an ideal set of immune receptors would recognize pathogens but ignore the proteins, DNA and other components that make up our own bodies. Self-reactive cells pose an immediate threat of autoimmunity. In higher organisms, multiple mechanisms of immunological tolerance eliminate or inactivate lymphocytes that bear receptors specific for autoantigens (reviewed by Goodnow et al., 2005). Nevertheless, some autoreactive lymphocyte clones escape these mechanisms and are present within the peripheral lymphocyte pool. One mechanism by which the pathogenic potential of these autoreactive clones is kept in check is through a dedicated lineage of regulatory Treg cells.

The coexistence of autoreactive and protective T cells was revealed by the multi-organ autoimmunity observed in lymphopenic (immune-deficient) recipient mice upon adoptive transfer of naïve $CD4^+$ T cells, and by the protection from autoimmune pathology upon co-transfer of a subset of $CD4^+$ T cells expressing IL-2 receptor α-chain (CD25) (Sakaguchi, 2004). Current evidence suggests that the $CD25^+CD4^+$ T cells are themselves self reactive, and that this property plays an essential role in the commitment to a Treg-cell lineage. Thus, self reactivity can be beneficial as part of a dedicated cellular mechanism preventing autoimmunity.

In addition to $CD25^+CD4^+$ Treg cells, other important self-reactive T cell sub-lineages have been identified. Prominent among these are cells that express a semi-invariant

TCR specific for conserved self ligands. These ligands, which are normally present at a low level, might be induced and serve as molecular signs of stress or infection. The best-characterized such T-cell sub-lineage is the CD1d-dependent natural killer T (NKT) cell. Mucosal invariant T cells, which are reactive with the MHC class-I-like molecule MR-1, are a second example of this type of T cell (Bendelac et al., 1997; Treiner et al., 2003). In contrast to $CD25^+CD4^+$ Treg cells, which have a dedicated suppressor function, NKT cells in some situations facilitate autoimmune pathology, but in others they are part of a protective mechanism.

It has long been believed that the thymus dictates one of three fates to developing T cells: death by neglect, positive selection or negative selection. Recent evidence hints at a 'fourth way' (Baldwin et al., 2004), in which selection by self agonists leads to the differentiation of T-cell subsets with an activated phenotype and specialized functions in the maintenance of self tolerance.

## B. T regulatory cells and autoimmune skin diseases

Immune responses need to be controlled and be turned down after completion, since without downregulation immune reactions are at high risk to become pathologic. In the past, $CD4^+CD25^+$ regulatory T cells have been shown to have a crucial role in dampening immune responses against pathogens and in maintaining immunological self-tolerance and immune homeostasis. In addition, Tregs represent an important cellular mechanism by which tumors evade immunosurveillance (Sakaguchi, 2005; Shevach, 2009). Hence, as key regulators of immune responses, Tregs have to be considered as therapeutic targets. Manipulation of numbers and/or suppressive activity of Tregs was shown to be useful in the treatment of various diseases, including autoimmune disorders, allograft rejection, graft-versus-host disease, and cancer (Shevach, 2009; Chen and Oppenheim, 2011). Accordingly, depletion of Tregs led to severe systemic autoimmunity and provoked immune responses to commensal bacteria in the intestine, causing inflammatory bowel disease (Singh et al., 2001; Ohkura et al., 2011), but on the other hand resulted in efficient antitumoral or antimicrobial immunity (Belkaid and Rouse, 2005). Moreover, antigenspecific expansion of Tregs was used to treat allergic diseases and establish immunological

tolerance to organ grafts, as well as prevent graft-versus-host disease after bone marrow transplantation (Ohkura et al., 2011).

Naturally occurring Tregs constitute 5–10 percent of total $CD4^+$ T cells in mice and healthy humans, are produced in the thymus as a distinct mature subpopulation of T cells, and are characterized by the expression of the forkhead family transcription factor, Foxp3, as well as characteristic cell surface markers, including a variety of co-stimulatory and co-inhibitory molecules (CD28, CTLA-4, inducible co-stimulator, etc.), TNF/TNF receptor family members, chemokine and Toll-like receptors, and a variety of immunosuppressive cytokines (IL-10, TGF-β, IL-35, etc.) (Lu and Rudensky, 2009; Shevach, 2009; Campbell and Koch, 2011; Chen and Oppenheim, 2011; Ohkura et al., 2011). In addition to naturally occurring thymic-derived $CD4^+CD25^+Foxp3^+$ Tregs, $Foxp3^-$ T cells with suppressive activity, including IL-10-secreting type 1 regulatory T cells or TGF-β-induced Th3 cells, have been described (Mills and McGuirk, 2004; Wu et al., 2007), which are thought to have a significant role in the maintenance of peripheral tolerance. However, little is known about the contribution of $Foxp3^-$ Tregs to ordinary immune responses and to sustaining self-tolerance.

Tregs exist in almost all tertiary tissues, and a particularly high number of Tregs has been detected in skin (Dudda et al., 2008; Tomura et al., 2010). To analyze the function of cutaneous Tregs, Dudda et al., 2008 reconstituted Foxp3-deficient scurfy mice with Tregs lacking the ability to migrate to the skin, which resulted in the rapid development of cutaneous inflammation and suggested that skin-resident Tregs have a key role in maintaining immune homeostasis at this site. Hence, it is conceivable that sterile cutaneous inflammatory and autoimmune diseases might be associated with impaired numbers or function of Tregs in lesional skin.

SLE is an autoimmune disease characterized by the loss of immune tolerance to self, leading to the activation and expansion of auto-reactive lymphocytes, as well as the production of autoantibodies and immune complexes targeting multiple organs, including the skin. It is generally accepted that the malfunction of the immune system in SLE is based on a dysregulation of T-cell tolerance. At least in murine models of SLE, central tolerance mechanisms are unaffected, the breakdown of tolerance seems

to emerge in the periphery, and thus abnormalities in Treg numbers and/or function have been suggested to contribute to T and B cell hyper-reactivity. Accordingly, most—but not all—studies revealed decreased numbers of CD4$^+$CD25$^{high}$ Tregs in peripheral blood from SLE patients; furthermore, the reduced Treg levels were found to be inversely correlated with disease activity (Lee et al., 2006; Mellor-Pita et al., 2006; Gerli et al., 2009; Buckner, 2010). However, these results could not be confirmed when Foxp3 was used as a marker for Tregs, suggesting that in SLE patients the expression of Foxp3 might be dissociated from the CD4$^+$CD25$^{high}$ Treg phenotype (Lin et al., 2007; Bonelli et al., 2008, 2009). Besides Treg numbers, the immunosuppressive activity of Tregs has been studied intensively in SLE patients, and several groups observed a defect in suppressor function, which correlated with disease progression (Alvarado-Sanchez et al., 2006; Venigalla et al., 2008; Miyara et al., 2009; Suen et al., 2009), whereas others could not confirm such functional deficiencies (Vargas-Rojas et al., 2008; Zhang et al., 2008). These contradictory results might be explained partially by the different methods used for Treg isolation and quantification of suppressive activity. Moreover, the majority of studies to assess Treg numbers and Treg function was carried out in peripheral blood, whereas there is little or almost no information on the aberration of Treg numbers and/ or function in lymphoid tissues or lesional skin. In SLE patients, disease activity varies between phases of relative remission and disease flares. Hence, it is conceivable that Treg expansion might be similarly inconsistent. Recently, Bonelli et al. (2009, 2010) identified a CD4$^+$Foxp3$^+$CD25$^-$ Treg subset with impaired suppressor function that was specifically induced in phases of active disease in SLE patients. Despite the large numbers of studies that have been performed, the role of Tregs during SLE development and progression remains elusive.

## V. Autoimmune Skin Diseases

The term 'autoimmune disease' refers to a varied group of more than 80 serious, chronic illnesses that involve almost every human organ system. It includes diseases of the nervous, gastrointestinal, and endocrine systems, as well as skin and other connective tissues, eyes, blood, and blood vessels. Prominent examples include

coeliac disease, diabetes mellitus type 1, SLE, Sjogren's syndrome, Churg-Strauss Syndrome, multiple sclerosis, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura, and RA. In all of these diseases, the underlying problem is similar - the body's immune system becomes misdirected, attacking the very organs it should protect.

Autoimmune diseases of the skin are a heterogeneous group of inflammatory disorders that are characterized by a chronic relapsing course and are associated with autoantibodies or cell-mediated immune response against self-antigens. These diseases affect all ages with a peak incidence during adulthood. Autoimmune diseases (collagen vascular disorders, vasculitides and autoimmune bullous diseases) are rare with an incidence of 1 to greater than 10 cases per million. Since these disorders may affect young patients and are chronic, prompt diagnosis and skilled long-term management can provide many years of improved quality of life.

A hallmark of autoimmune skin diseases are distinct clinical and immunological features that help to establish the diagnosis. Collagen vascular skin disorders – the best known of which is SLE – are characterized by fibrosis (skin thickening), myopathy (muscle damage), rashes and UV sensitivity. They are commonly associated with ANA. The fine specificity of the ANA profile helps to distinguish the different clinical sub-types and may be of prognostic value. Autoimmune bullous skin disorders may present with wide areas of blistering causing problems similar to burns. The most serious form – PV – was almost invariably fatal prior to corticosteroid therapy. Bullous pemphigoid is the most common form and is seen in primarily in the elderly. They are diagnosed by the serum autoantibody profile against defined autoantigens, which are adhesion molecules critical for the skin integrity. Vasculitides have been characterized based on the size of affected vessels and immunological parameters. Both collagen vascular diseases and vasculitides may affect internal organs such as lung heart, liver kidneys, joints and central nervous system.

The causes of all these disorders are poorly understood. Even though the target antigens and autoantibodies have been extensively characterized, the triggers of the aberrant response remained difficult to identify. Environmental agents such as drugs, infection, and UV radiation may catalyze the reactions with an exuberant host

immune response then attacking similar self-antigens (molecular mimicry). Overall, there is strong evidence that autoimmunity is linked to a loss of self-tolerance that may be due to an imbalance between regulatory and effector T lymphocytes. Both SSc and SLE are more common in women.

Treatment of these disorders is commonly based on a combination of systemic corticosteroids and immunosuppressive drugs, which have greatly improved their overall prognosis. The crux of therapy is balancing the benefits of damping the immune response against the inevitable side effects. Novel therapeutic options such as immuno-adsorption, intravenous immunoglobulins and the B cell depleting antibody, rituximab have been successfully employed in several autoimmune diseases of the skin. The exact mode of action of these potent anti-inflammatory treatments remains to be fully understood. Supportive therapies such as physiotherapy and rehabilitation are particularly important for these chronic disorders.

## A. Pemphigus

Autoimmune bullous diseases are a group of cutaneous disorders characterized by skin blistering or erosions as a result of development of autoimmunity. It can be classified according to the anatomical sites of blister into two types: intraepithelial and sub-epidermal. The term "pemphigus" refers to intraepithelial blistering skin diseases. The term "pemphigoid" refers to blistering diseases occurring at the dermo-epidermal junction in general, although not every sub-epidermal bullous disease bears the term "pemphigoid" in the nomenclature.

Pemphigus (from the Greek *pemphixss*, meaning bubble or blister) is a blistering disease involving the skin and mucous membrane. The disease is characterized histologically by acantholysis which means loss of adhesion between keratinocytes, and immuno-pathologically by the presence of immunoglobulin directed towards the cell surface of keratinocytes (Stanley, 1999).

With extensive research, most of the target antigens of these disorders have been characterized. It is amazing to see that earlier disease classification by clinical features and pathological findings alone did have a molecular basis, as distinct antigens are targets for different entities in most of the subgroups of autoimmune

bullous diseases. The antigens of pemphigus are found in desmosomes between keratinocytes, which are organelles mediating intercellular adhesion together with tissue morphogenesis and differentiation. On the other hand, the antigens of sub-epidermal bullous diseases are found in the basement membrane zone at the dermo-epidermal junction. Research in these target antigens has led to a better understanding of their biologic functions, advance in disease diagnosis, monitoring of disease activity, and can be potentially employed for antigen specific therapy (Bickle et al., 2002).

Epidermal cells possess structures that are involved either in cell–cell (desmosomes) or in cell–matrix adhesion (hemidesmosomes-anchoring fibrils complex). Whenever autoantibodies target proteins in these adhesion structures, intra- or sub-epidermal separation often occurs, and clinical signs of an autoimmune blistering skin disease usually develop (Fassihi et al., 2006). In the last 30 years, the identification of antigens targeted by circulating autoantibodies has helped reshape the classification of autoimmune blistering skin diseases in humans. Based on clinical signs, histopathology and immunological characteristics, several entities are now well recognized. For example, the most common diseases associated with autoantibodies against epidermal basement membrane antigens are bullous pemphigoid (target: collagen XVII), mucous membrane pemphigoid (laminin-5, collagen XVII, integrin, α-6 β-4) and epidermolysis bullosa acquisita (collagen VII) (Fassihi et al., 2006). Similarly, autoantibodies targeting desmosomal proteins in keratinocytes may result in blistering diseases of the pemphigus group. This group encompasses both deep (Pemphigus vulgaris (PV): desmoglein-3±desmoglein-1; paraneoplastic pemphigus: desmoglein-3, plakins) and superficial variants (pemphigus foliaceus (PF): desmoglein-1; IgA pemphigus, desmocollin-1 and 3) (Fassihi et al., 2006).

**Figure 4:** A severe case of Pemphigus vulgaris. Flaccid bullae have broken down to form erosions and crusts.
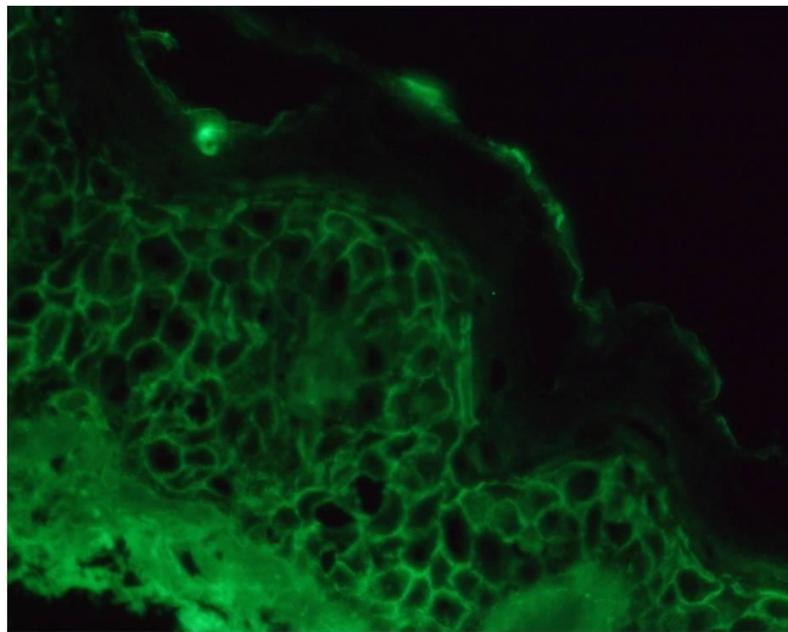


**Figure 5:** Direct immunofluorescence microscopy performed on a skin biopsy specimen obtained from a patient with pemphigus vulgaris detects immunoglobulin G in intercellular 'fishnet' pattern.

**Table 1**: Epidemiology, clinical features and risk of pemphigus vulgaris and pemphigus foliaceus

| Bullous diseases | Epidemiology | Clinical features | Risks |
|---|---|---|---|
| **Pemphigus vulgaris** | Rare, equal in men and women, onset at 40 to 60 years of age, more common in Jewish or Mediterranean descendants | Flaccid blisters or crushed erosions, located on head, upper trunk, intertriginous areas, and mucosa; often begins on oral mucosa, may lead to hoarseness; skin is usually painful and Nikolsky's sign positive | May be fatal if untreated, risk of infection in treated and untreated cases |
| **Pemphigus foliaceus** | Rare and sporadic worldwide, equal in men and women, onset at about 50-60 years, no predominance in Jewish or Mediterranean descendants | Characteristic lesions that are scaly, crusted erosions, often on an erythematous base. No clinically apparent mucosal involvement is present even with widespread disease | Tends to persist for months to years, may coexist with thymoma, myasthenia gravis, lupus erythematosus, and other autoimmune bullous diseases |

Systemic corticosteroids remain the treatment of choice for pemphigus as they are both effective and capable of inducing a rapid remission. However, adverse effects of corticosteroids are both time- and dose-dependent (Buchman, 2001). They include weight gain, diabetes, hypertension, glaucoma, cataracts, osteoporosis, avascular necrosis, peptic ulcer disease, adrenal insufficiency, electrolyte and lipid abnormalities, psychosis, immunosuppression, and increased susceptibility to infections (Buchman, 2001). Adjuvant therapies are, therefore, used to provide a steroid-sparing effect. As these treatments typically have a slower onset of action (i.e., 4-6 weeks), they are most beneficial as maintenance therapies. Conventional adjuvants include various immunosuppressive agents such as azathioprine, mycophenolate mofetil, methotrexate, cyclophosphamide, chlorambucil and cyclopsorine, as well as anti-inflammatory agents such as gold, dapsone, colchicine and a variety of tetracycline antibiotics (Yeh et al., 2005; Dick and Werth, 2006; Mydlarski et al., 2006). Unfortunately, these medications are often associated with significant toxicities and must be used with caution. Though the majority of patients

will ultimately respond to conventional therapies, few patients develop recalcitrant disease.

## B. Scleroderma

Scleroderma is a systemic autoimmune disease with a heterogeneous and complex phenotype that encompasses several distinct subtypes. The disease has an estimated prevalence of 276 cases per million adults in the United States (Mayes, 1998; Mayes et al., 2003). Median age of onset is 45 years of age with the ratio of females to males being approximately 4:1. Scleroderma is divided into distinct clinical subsets. One subset is the localized form, which affects skin only including morphea, linear scleroderma and eosinophilic fasciitis. The other major type is systemic sclerosis (SSc) and its subsets. The most widely recognized classification system for SSc divides patients into two subtypes, diffuse and limited, a distinction made primarily by the degree of skin involvement (Leroy et al., 1988). Patients with SSc with diffuse scleroderma (dSSc) have severe skin involvement (Medsger, 2001) often characterized by more rapid onset and progressive course with fibrotic skin involvement extending from the hands and arms, trunk, face and lower extremities. Patients with SSc with limited scleroderma (lSSc) have fibrotic skin involvement that is typically limited to the fingers (sclerodactyly), hands and face. Some patients in the limited subset develop significant pulmonary arterial hypertension, pulmonary fibrosis or digital ischemia/ulcerations. Although there are certain disease characteristics that differentiate these two groups, some of the severe vascular and organ manifestations occur across groups and are the cause of significant morbidity and mortality (Masi, 1988).

Skin thickening is one of the earliest manifestations of the disease; it remains the most sensitive and specific finding (Committee SfSCotARADaTC, 1980) and is one of the most widely used outcome measures in clinical trials (Seibold and McCloskey, 1997; Clements et al., 1990; 2000). Several studies have demonstrated that the extent of skin involvement directly correlates with internal organ involvement and prognosis in SSc patients (Barnett et al., 1988; Scussel-Lonzetti et al., 2002; Shand et al., 2007). Diffuse scleroderma can cause musculoskeletal, pulmonary, gastrointestinal, renal and other complications (Benedek, 1997). The first joint symptoms that patients with

scleroderma have are typically non specific joint pains, which can lead to arthritis, or cause discomfort in tendons or muscles (Benedek, 1997). Joint mobility, especially of the small joints of the hand, may be restricted by calcinosis or skin thickening (Valentini G, Black, 2002). Patients may develop muscle weakness, or myopathy, either from the disease, or its treatments (Olsen et al., 1996). Some impairment in lung function is almost universally seen in patients with diffuse scleroderma on pulmonary function testing (Steen, 2005); however, it does not necessarily cause symptoms, such as shortness of breath. Other pulmonary complications in more advanced disease include aspiration pneumonia, pulmonary hemorrhage and pneumothorax (Benedek, 1997). Diffuse scleroderma can affect any part of the gastrointestinal tract (Sallam et al., 2006). The most common manifestation in the esophagus is reflux esophagitis, which may be complicated by peptic stricturing, or benign narrowing of the esophagus (Rose et al., 1998). This is best initially treated with proton pump inhibitors for acid suppression (Hendel et al., 1992), but may require bougie dilatation in the case of stricture (Sallam et al., 2006). Renal involvement, in scleroderma, is considered a poor prognostic factor and frequently a cause of death (Ruangjutipopan et al., 2002).

Scleroderma is a rare disease, and most epidemiological information has been obtained from patients treated in health clinics with defined focuses. The death rate from the disease is low at young ages and increases slowly with advancing age. The ratio of women to men with scleroderma decreases with age, from 7:1 in early adulthood to about 3:1 in the fifth decade of life. Distribution of the disease is apparently unrelated to urban or rural life, but may be somewhat higher in US blacks, and varies among countries. It appears to be more common in the South Atlantic and East South Central areas of the US.

Systemic scleroderma is a generalized disorder of small arteries, micro vessels and connective tissue of unknown origin with the highest incidence occurring between 45 and 55 years of age (Steen and Medsger, 1990) and with a frequency of three to eight times higher in females than in males (Medsger, 1994). In SSc different types of antibodies are produced, including anti-centromere and anti-topoisomerase antibodies (Artlett et al., 1998). Major disease mechanisms are the prominent vascular changes

due to endothelial-cell damage and proliferation of sub endothelial connective tissue. The widespread damage of small arteries and micro vessels results in increased deposition of collagen and other matrix elements, leading to thickening and fibrosis of the skin, involvement of synovia and visceral fibrosis (LeRoy et al, 1988).

There is no cure for every patient with scleroderma, though there is treatment for some of the symptoms, including drugs that soften the skin and reduce inflammation. Some patients may benefit from exposure to heat (Oliver and Winkelmann, 1989). The skin tightness may be treated systemically with methotrexate and cyclosporin (Zandman-Goddard et al., 2005). Active alveolitis is often treated with pulses of cyclophosphamide, often together with a small dose of steroids. The benefit of this intervention is modest (Tashkin et al., 2006; Hoyles et al., 2006). Pulmonary hypertension may be treated with epoprostenol, bosentan and possibly aerosolized iloprost (Zandman-Goddard et al., 2005).

## C. Systemic Lupus Erythematosus

Systemic lupus erythematosus or lupus is a chronic systemic autoimmune disease. As is true in other related entities, its etiology is unknown. Multiple defects in the immune system of patients with this condition have been described. It can be fatal, though with recent medical advances, fatalities are becoming increasingly rare. SLE can affect any part of the body, but most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called flares) alternating with remissions. Lupus can occur at any age, and is most common in women, particularly of non-European descent [Lupus Foundation of America (www document), 2009]. Symptoms of SLE vary widely, it often mimics or is mistaken for other illnesses because the symptoms wax and wane unpredictably. Diagnosis can be elusive, with patients sometimes suffering unexplained symptoms and untreated SLE for years. Common initial and chronic complaints are fever, malaise, joint pains, myalgias, fatigue and temporary loss of cognitive abilities. Because they are so often seen with other diseases, these signs and symptoms are not part of the diagnostic criteria for

SLE. When occurring in conjunction with other signs and symptoms, however, they are considered suggestive (Lupus (www document), 2009).

Morphological changes typical for SLE are present in almost every organ. Nevertheless, analyses of skin and kidney lesions are of major importance for histopathological diagnosis of SLE. Less important lesions include changes that are present on serous surfaces, joints, cardiovascular system, central nervous system, lungs etc (American College of Reumathology ad hoc Committee on SLE in adults, 1999; Petrovic, 2000; Tatic et al., 2000). Skin lesions in SLE are characterized by following changes: hyperkeratosis and parakeratosis atrophy of Malpighi's layer, liquefactive degeneration of basal epidermal layer, acute necrotizing vasculitis with lymphocytic perivascular and periadnexal islet-like infiltration, connective tissue edema, vasodilatation and extravasation of erythrocytes (Barnhill, 1998).



(a)      (b)

(c)
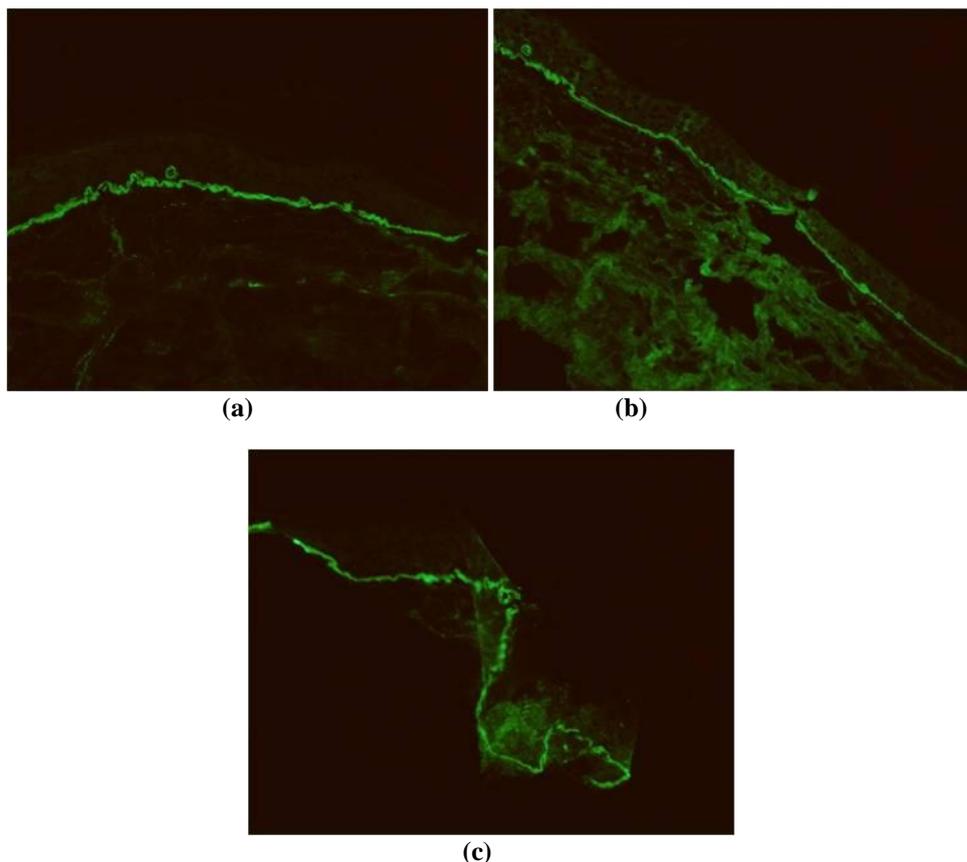
**Figure 6:** Direct immunofluorescence using fluorescent antibodies to C3, IgG and IgM on a skin biopsy from a SLE patient. Microscopic image showing linear staining along the dermal-epidermal junction for C3 (a); a band-like accumulation of IgG along the basement membrane (b); and greater positivity for IgM with granular pattern (c).

SLE is characterized by the presence of antinuclear antibodies. Nuclear antigens become recognizable by the immune system which causes an immune response and subsequent tissue damage. Since these nuclear antigens are not limited to the skin, all tissues and organ systems are possible targets of the autoimmune reaction (joints, skin, bone marrow, kidney, etc) (Rothfeild, 1993). SLE is triggered by environmental factors that are unknown in people with certain combinations of genes in their immune system. The likely environmental triggers include ultraviolet light, drugs, and viruses. These stimuli cause the destruction of cells and expose their DNA, histones, and other proteins, particularly parts of the cell nucleus. Because of genetic variations in different components of the immune system, the response elicited by them attacks nuclear-related proteins and produces antibodies against them. Ultimately, these antigen-antibody complexes damage blood vessels in critical areas of the body (e.g. the glomeruli of the kidney); such attacks are the cause of SLE. Researchers are now identifying the individual genes, the proteins they produce, and their role in the immune system. Each protein is a link on the autoimmune chain, and researchers are trying to find drugs to break each of those links (Rahman and David, 2008; Crow, 2008; Hom et al., 2008). Patients with active SLE exhibit a quantitative defect of natural $CD4^+CD25^{+high}$ Treg cells. A decrease in natural $CD4^+CD25^{+high}$ Treg cells correlates with the clinical seriousness of the skin lesions (Crispin et al., 2003). This defect is absent during the remission of the disease. Relapses of SLE are therefore associated with the global decline of these cells and do not represent some phenomenon of tissue redistribution (Miyara et al., 2005).

Due to improved detection of mild disease, the incidence of SLE has nearly tripled in the last 40 years (Uramoto et al., 1999). Estimated incidence rates in North America, South America, Europe and Asia range from about 15 (New Zealand) to 254 (N. Ireland) per 100,000 per year (Rus et al., 2002; Danchenko et al., 2006). A few subsets of SLE have been defined (Cervera et al., 1993). Two of these subsets, sub acute cutaneous and neonatal lupus, are associated with anti-Ro and HLA-DR3 (Sontheimer et al., 1981).

The disease appears to be more common in urban than rural areas (Petri, 2002). The prevalence of SLE is higher among Asians, Afro-Americans, Afro-Caribbeans, and

Hispanic Americans compared to Americans of European descent in the United States, and among Asian Indians compared to Caucasians in Great Britain (Serdula and Rhoads, 1979; Hochberg, 1985; Rus et al., 2002). In comparison, SLE occurs infrequently in Blacks in Africa (Symmons, 1995). In New Zealand, the prevalence and mortality of SLE are higher in Polynesians than in Caucasians (Hart, 1983). The increased frequency of SLE among women has been attributed to an estrogen hormonal effect (Cooper et al., 1998; Costenbader et al., 2007). In support of the potential role of estrogens in predisposing to SLE, the Nurse's Health study showed that women with early menarche, or treated with estrogen-containing regimens, such as oral contraceptives or postmenopausal hormone replacement therapies, have a significantly increased risk for SLE (hazard ratios of 1.5 to 2.1 (Costenbader et al., 2007)). Other possibilities for female predisposition include: X-inactivation, imprinting, X or Y chromosome genetic modulators, differential methylation of DNA and acetylation of histones bound to DNA, intrauterine influences, chronobiologic differences, pregnancy, and menstruation (Ballestar et al., 2006; Lockshin, 2006).

Sixty-five percent of patients with SLE have disease onset between the ages of 16 and 55 (Rothfield, 1981). Of the remaining cases, 20 percent present before age 16 (Schaller, 1982), and 15 percent after age 55 (Ballou et al., 1982). Median ages at diagnosis for white females range from 37 to 50 years, in white males from 50 to 59, in black females from 15 to 44 and in black males from 45 to 64 (Rus et al., 2002).

Lupus is treatable symptomatically, mainly with corticosteroids and immunosuppressants, though there is currently no cure. Survival in patients with SLE in the United States, Canada, and Europe is approximately 95 percent at 5 years, 90 percent at 10 years, and 78 percent at 20 years (Harrison's Internal Medicine, 17th ed. Chapter 313). Mild or remittent disease can sometimes be safely left untreated. If required, nonsteroidal anti-inflammatory drugs and antimalarials may be used. Disease-modifying antirheumatic drugs (DMARDs) are used preventively to reduce the incidence of flares, the process of the disease, and lower the need for steroid use; when flares occur, they are treated with corticosteroids. DMARDs commonly in use are antimalarials and immunosuppressants (e.g., methotrexate and azathioprine). Hydroxychloroquine (trade name Plaquenil) is an FDA-approved antimalarial used

for constitutional, cutaneous, and articular manifestations, while cyclophosphamide (trade names Cytoxan and Neosar) is used for severe glomerulonephritis or other organ-damaging complications.

**Table 2:** Common systemic autoimmune diseases and their target antigens with organs affected.

| Autoimmune Diseases | Target antigen(s) | Organ(s) affected | Consequences |
|---|---|---|---|
| **Systemic Lupus Erythematosus** | DNA, Histones, Ribosomes, small nuclear or cytoplasmic ribonucleoproteins | Skin, Kidneys, Joints/Tendons/ Muscles, Nerves, Lungs, Heart valves, GI tract | Glomerulonephritis, Vasculitis, Skin lesions |
| **Scleroderma** | Kinetochore of the chromosomes, HLA-DR5, Topoisomerase-I | Skin, Heart, Kidney, Lungs, Intestines | Hardening and scarring of skin, Skin itching, Chronic renal failure, Musculoskeletal, Pulmonary and Gastrointestinal complications |