

## Invited Review

# Molecular and Cellular Mechanisms Associated with Autoimmune Diseases

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**Abstract:** Evidence points to increases in the incidence and prevalence of several autoimmune diseases in the United States. As a result, the cost to public health from clinical management of autoimmune conditions is on the rise. The initiation and progression of autoimmune disturbances involves both genetic and environmental factors. Deficiencies in important proteins that normally participate in maintaining checks and balances within the internal milieu may render an individual prone to developing autoantibodies. Structural abnormalities or decline in normal levels of the pentraxins (serum amyloid-A protein, the acute phase proteins, complement, and C-reactive proteins) have been shown to induce autoimmunity. Irregular transmission of information arising from multiple signal transduction pathways typically associated with the serine/threonine cascade routes of mitogen activating phosphorylation kinases, has also been found to induce autoimmunity. The kind of ligand/receptor interactions drives physical recruitment of different signals within the lymphocyte; these links define the quality and quantity of subsequent immune responses. CD95 or the Fas/Apo-1 and its ligand CD95L participate in regulating lymphocyte populations and therefore influence various aspects of immune responses. Mutational abnormalities resulting from synthesis of proteins by the CD95 and/or its ligand CD95L may result in alterations in the apoptotic pathways. Apoptosis may be completely inhibited, activated or partially stimulated. Modulation of apoptosis may lead to accumulation of self-antigens. Subsequently the immune system may be stimulated to react against self-molecules through lymphatic hyperplasia. This process may end up in proliferative disorders and enhanced susceptibility to autoimmune syndromes. This paper deals with mechanisms of autoimmunopathogenesis at the cellular and molecular levels. Emphasis is laid on the role of T and B cell receptor/ligand interactions, functions and malfunctions due to structural and quantitative alterations in T- B- cell cluster of antigen determinants. Genetically susceptible patients who develop spontaneous autoimmune diseases are examined and the etiological factors implicated in the initiation and subsequent dissemination of autoimmune diseases is discussed.

**Key words:** self-tolerance, T cell receptor, B cell receptor, autoimmunopathogenesis, apoptosis

## Introduction

T lymphocytes are credited with determining the functional outcome of immune responses and form

important components of the adaptive immune system. The specificity of T-cell-mediated immune responses is dependent upon the T-cell receptor (TCR) on CD4 helper or CD8 cytotoxic T cells. The respective TCRs

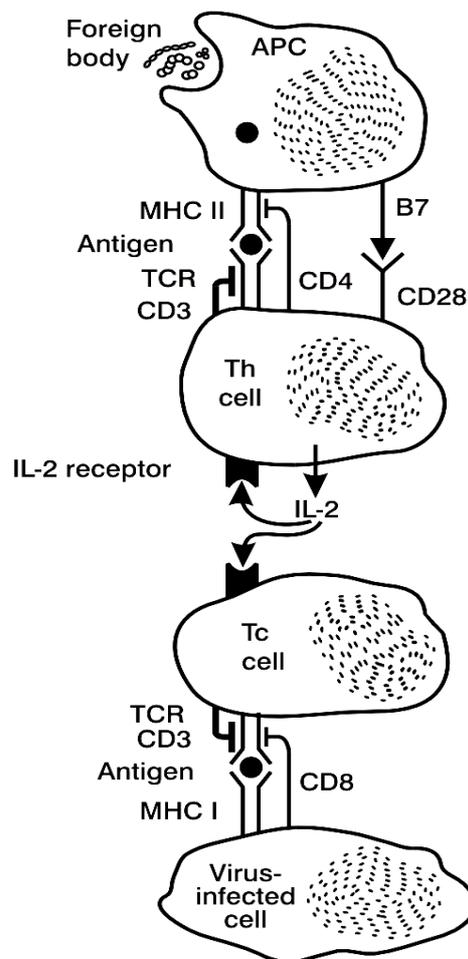
identify a peptide in association with major histocompatibility complex (MHC) class II or class I molecules on target cells. CD4 T cells control ongoing immune responses by regulating the functions of other immune cells, such as B cells, whereas CD8 T cells directly attack and kill target cells. In both instances, T-cell activities in the course of immune responses are critical in initiating and regulating T-cell-mediated immune functions and even more so in many individuals prone to develop autoimmunity. Various mechanisms are known to control and halt an ongoing immune response and defects in TCR signaling invariably impair T-cell development and/or cause deviation of T-cell function [1, 2]. Costimulatory molecules play important roles in these pathways [3]. Balancing of positive and negative signaling costimulatory pathways dictates the fate of individual T cells and the immune response (Figures 1 and 2).

Most of the cytokine receptors whose ligands regulate proliferation and differentiation in the hematopoietic and some hormones as well as the antigen-specific receptors on T- and B-lymphocytes fall into a class of heterogenous receptors that lack an obvious catalytic domain. Many of these receptors operate via tyrosine kinases that phosphorylate various target proteins when the receptor binds its ligand. They belong either to the Src family of nonreceptor protein kinases or the Janus family of nonreceptor protein kinases [4]. Their kinase domain is however encoded by a separate gene from the receptor tyrosine kinases and is noncovalently associated with the receptor's polypeptide chain. Activation of these family members occurs via ligand-induced dimerization just as the other tyrosine kinases. There are about eight members of the Src family of nonreceptor protein tyrosine kinases, namely Src, Yes, Fgr, Fyn, Lck, Lyn, Hck, and Blk. They possess two highly conserved noncatalytic domains, called SH2 and SH3 (for Src homology regions 2 and 3, because they were first found in the Src protein) located on the cytoplasmic face of the plasma membrane through their interaction with transmembrane receptor proteins and, in part, through covalently attached lipid chains. The SH2 domains identify phosphorylated tyrosines and enable proteins that contain them to bind to the activated receptor tyrosine kinases (RTKs), as well as to other intracellular signaling proteins that have been transiently phosphorylated on tyrosines. The function of the SH3 domains is still not clarified but is believed to bind other proteins in the cells that lack the SH3 domain. Various types of the group associate with different receptors and phosphorylate overlapping, but distinct, sets of target proteins. Some receptors are protein tyrosine phosphatases (PTPs) able to dephosphorylate tyrosine residues rapidly from selective phosphotyrosines on particular types of proteins [5, 6].

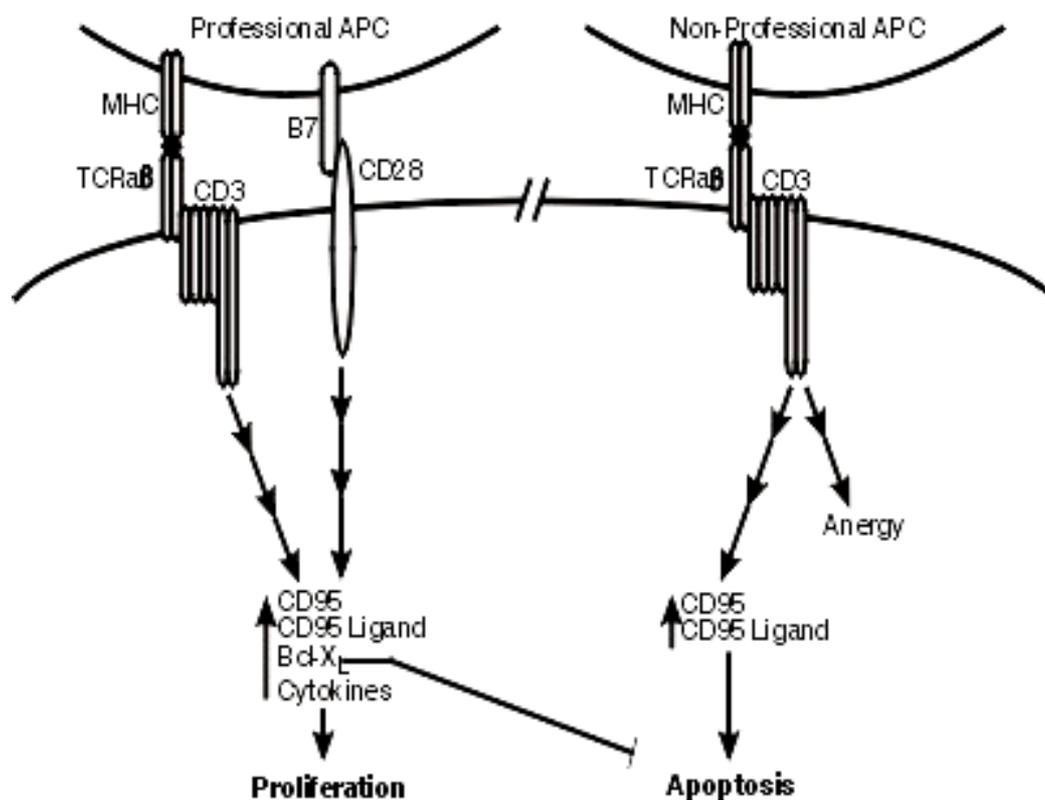
Different types of PTPs are known to have high specific activities that make their tyrosine phosphorylation actions very short-lived. The level of phosphorylation in resting cells is also very low. They

play specific roles in cell signaling and in the cell cycle. The cluster of differentiation antigen 45 (CD45) is one example of a regulated protein tyrosine phosphatase (RPTP) bound to the surface of leucocytes that plays an important role in the activation of both T- and B-lymphocytes. It is a single-pass transmembrane glycoprotein whose phosphatase activity can be halted on dimerization and thus associated with autoimmune induction leading to pathological consequences both in humans and in mice [7-13]. Several of the genes that encode the proteins in the intracellular signaling cascades that are activated by receptor tyrosines were initially isolated as oncogenes in cancer cells or tumor viruses. Inappropriate activation of these signaling proteins causes a cell to proliferate excessively [4, 8, 13-16]. Adaptor proteins located between the signal generating tyrosine kinases and more nonspecific cellular control circuitry, behave as primary mechanisms regulating the enlistment of downstream signaling pathways after ligand-binding to the TCR [15, 17]. For instance Lck and Fyn phosphorylate TCR to provide binding sites for some soluble intracellular adaptor polypeptides.

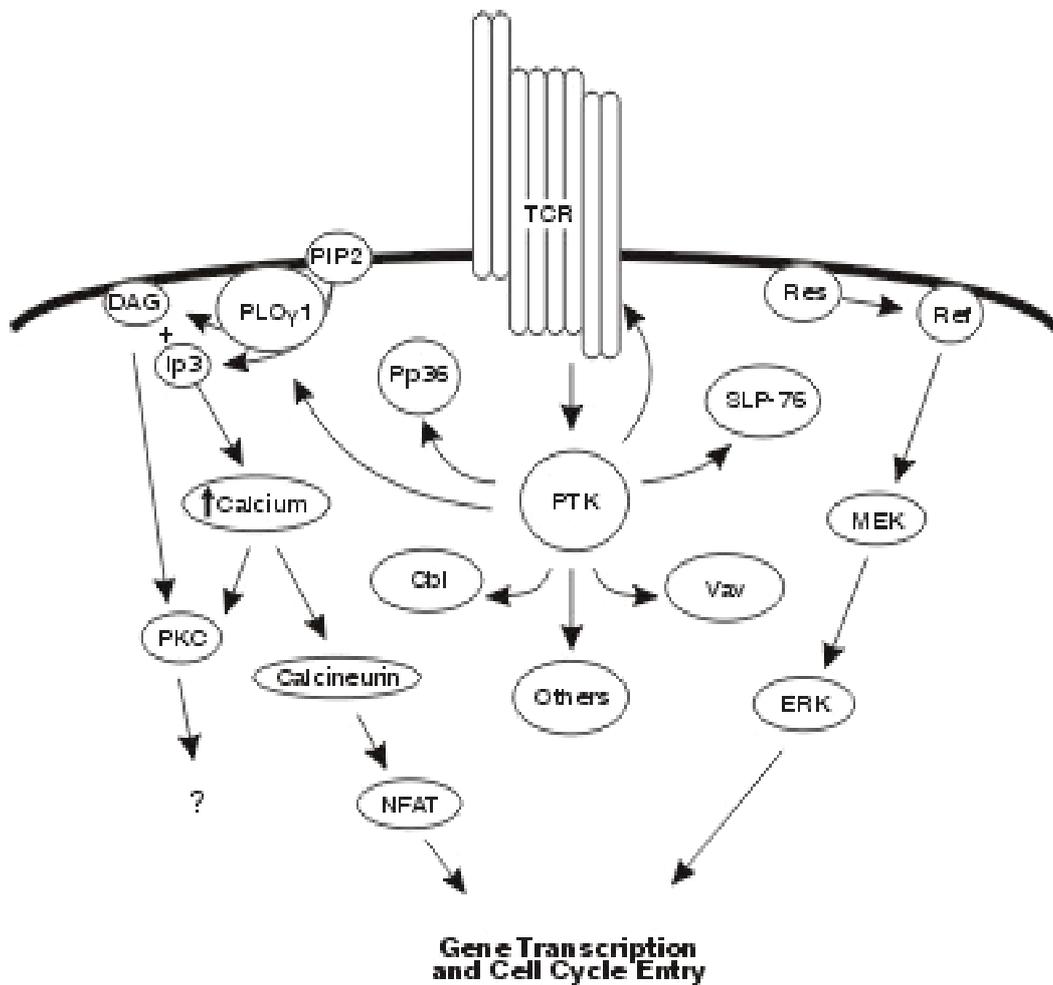
These adaptor proteins have interactive domains and sites for tyrosine phosphorylation but lack enzymic activity. Several adaptor proteins such as Sch, p36-38, and Cbl are known to interact with TCR. However Grb2 is the best characterized involved in the activation of Ras. SLP-76 is also an adaptor protein that associates with Grb2 in T cells [17, 18]. It possesses several potential tyrosine phosphorylation sites, an SH2 domain, and proline-rich area that may bind to SH3-containing proteins. TCR binding stimulates SLP-76 phosphorylation; and increased expression of SLP-76 enhances coupling of the TCR to the nuclear factor of activated T cells (NF-AT) transcription factor, an important regulatory event in IL-2 expression; Figure 3. Several other adaptor proteins are believed to competitively bind to different domains of Grb2; and binding to one domain of Grb2 influences which proteins subsequently bind to other domains [17-19]. Thus the TCR has numerous pathways of activation through the adaptor proteins. Subtle changes in the presence of adaptor proteins, their relative affinities for receptor polypeptides, and time needed for binding to related ligands may greatly affect the nature of signaling through the TCR. This means that alterations in the phosphorylation of each immunoreceptor tyrosine-based activation motif (ITAM) in the course of T cell activation may generate a mixture of signaling. In this way the TCR signal transduction apparatus is able to send out multiple independent signals. T cell-specific adaptor protein (TSAd) is known to be involved in the assembly of intracellular signaling complexes in T cells and also participate in the induction of T cell interleukin 2 secretion and proliferation. Deficiencies in some of these CD proteins involved in T lymphocyte activation and function have been associated with development of autoimmunity [15].



**Figure 1:** Th/Tc cell activation - Antigen (foreign body) is phagocytosed by antigen presenting cell (APC). Antigen is presented on MHCII and seen by TCR on Th cell. “Costimulatory signal” is induced by interaction of B7 and B28. Activated Th cell produces IL-2 and  $\gamma$  interferon. Tc cell Activation: Endogenously synthesized (viral or self) proteins are presented on MHCI and seen by TCR on Tc cell to kill virus-infected cell. Autocrine and paracrine effects of IL-2 as well as cell-cell contact stimulation are illustrated.



**Figure 2:** Pathways of T-cell stimulation [2] – The role of costimulation in delaying the onset of apoptosis induced cell death (AICD). Ligation of the T cell receptor in the presence or absence of costimulation results in increased expression of both CD95 and its ligand. However when professional antigen-presenting cells (APC) that express the B7 surface antigen stimulate T cells via CD28-mediated costimulatory signals, augmentation of TCR-induced cytokine production goes on for several days. The cells become susceptible to CD95/CD95 ligand-mediated apoptosis. In contrast when T cells are stimulated directly through the TCR in the absence of B7 surface antigen costimulation they are signaled to either enter a state of unresponsiveness (anergy) or are induced to undergo apoptosis due to interaction of CD95 with its ligand.



**Figure 3:** T-cell receptor signaling pathways - Protein tyrosine kinase activation couples the TCR to downstream signaling cascades. Activation of the phosphatidylinositol 2<sup>nd</sup> messenger pathway follows the stimulation of phospholipase C-gamma-1 (PLCγ1), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 induces the release of intracellular calcium stores and the activation of calcium sensitive enzymes. Calcineurin is a Ca<sup>2+</sup>-dependent serine/threonine phosphatase that dephosphorylates the cytosolic form of nuclear factor of activated T cells (NFAT), resulting in its nuclear translocation and activation of gene transcription through the NFAT response element. Protein kinase C (PKC) activation by DAG and calcium flux results in the serine/threonine phosphorylation of PKC substrates. Ras activation results in the recruitment of Raf to the plasma membrane, which activates the dual specific serine tyrosine kinase MEK, which in turn activates ERK, a member of the MAP kinase family. Activated ERK phosphorylates transcription factors that, along with other TCR-induced transcription factors, activate the transcription of new genes; Vav, Cbl, SLP-76, pp36, and “others” are shown as molecules that may link PTK activation to distal signaling pathways [2].

Different ligands can also alter the signaling properties of the B cell antigen receptor (BCR) similar to the TCR. B-lymphocytes on encountering antigen differentiate to form plasma cells capable of synthesizing and secreting large amounts of antibody with a single specificity. Membrane-bound immunoglobulins (mIgs) have only 3 cytoplasmic disposed amino acids. Like the TCRs, therefore BCRs do not possess the capacity to transmit signals alone. The cytoplasmic tails of Ig $\alpha$  and Ig $\beta$  retain the signaling apparatus of the BCR. Both are homologous and functionally equivalent to the ITAM-containing components. Lipopolysaccharide (LPS), a B cell mitogen binds to serum LPS-binding protein (LBP) at physiological concentrations before interacting with CD14 on macrophages or neutrophils. CD14 is a glycosylphosphatidylinositol-linked (GPI) protein that lacks an intracellular domain. LPS-LBP binding to CD14 (LPS-LBPCD14) causes a rapid phosphorylation of various proteins on tyrosyl residues [20]. Ligation of the BCR and LPS binding to CD14 both stimulate Src and Syk family of PTKs like T cells: the Btk, Fyn, Lyn, and ZAP-70 for BCR and for LPS/CD14 interaction the Hck, Fgr, Lyn and p38 are involved in signal transduction [21-24]. Phosphorylation of the ITAMs of Ig $\alpha$  and Ig $\beta$  and the CD14/LPS receptor conducts subsequent signaling events downstream. This involves the Ras pathway. Stimulation of the TCR, BCR and macrophage through LPS binding to CD14 require activation of three known pathways used by the mitogen-activated protein kinase (MAPK) family cascades [23, 24].

The Ras proteins are among a superfamily of monomeric GTPases composed of two subfamilies: 1) Rho and Rac proteins that convey relay-signals from cell surface receptors to the actin cytoskeleton; and 2) the Rab family involved in regulating the flow of intracellular transport vesicles [2, 25, 26]. Through phenyl groups the Ras proteins are covalently bound to the cytoplasmic face of the plasma membrane and relay signals from RTPKs to the nucleus to initiate cell proliferation or differentiation. These proteins undergo conformational changes on binding to GTP; this process results in their stimulation to behave as switches in relaying signals between cell surface and the nucleus. The Ras proteins are in turn regulated by GTPase-activating protein (GAP) and guanine nucleotide releasing proteins (GNRPs). Active RTKs directly bind GAPs and indirectly associate with GNRPs. The indirect binding to GNRPs is normally responsible for driving Ras into its active GTP-bound state. Activated Ras sends signals downstream by stimulation of a serine/threonine phosphorylation (STPs) cascade [25-27]. However signals initiated by Ras from the cytoplasmic face to the nucleus are short-lived because phosphorylations are rapidly reversed by tyrosine PTPs and hydrolysis of GTP-bound Ras to GDP. These short-lasting signals are nevertheless converted to longer-lasting ones to propagate cell proliferation or differentiation. This relay pathway incorporates multiple, interacting cascades of

STPs. Several serine/threonine kinases are involved in these cascades but MAPK family of proteins play crucial role in inducing these longer lasting signals [27].

The MAPKs, also called extracellular-signal regulated kinases (ERKs), are STP kinases consisting of at least five members usually activated by a wide range of extracellular proliferation- and differentiation-inducing signals (Figure 3). Some of these signals stimulate RTPs and others activate G protein-linked receptors. The MAPKs are unusual in the sense that they need the phosphorylation of both a threonine and a tyrosine, separated by one amino acid. MAPK-kinase is itself stimulated by STP catalyzed by MAPK-kinase-kinase believed activated by its binding to activated Ras [27-29]. The complexity of TCR- BCR/ligands interactions may help explain why malfunctions or deficiencies of these molecules can result in the generation of signals that are potentially pathogenic and capable of inducing autoimmunity as well as cancer from proliferative disorders.

Epidemiological data from U.S. census clearly predicts high prevalence of autoimmune syndromes among numerous types of diseases, (Table 1); and point to widespread among the population more than envisaged [30]. Besides only some individuals exposed to environmental chemicals develop chemical intolerance. This fact directs attention to the possibility that genetic factors could influence the degree of susceptibility. Several data suggest that an abnormal or enhanced cholinergic system could be one avenue of predisposition to autoimmunity, asthmatic tendencies [31] and as well as tolerance inductions to substances [32]. This is evidenced by differences in immune reactions exhibited by strains of rats that inherently react with hyper- or hyporesponses to cholinergic agonists [33, 34]. Individuals suffering from multiple chemical sensitivity (MCS) or chemical intolerance (CI) regularly complain of symptoms identical to those patients experiencing chronic fatigue syndrome and or asthma [35, 36]. Similarly humans accidentally and occupationally exposed to mercury show signs of acute and chronic behavioral effects such as cognitive (eg., memory and concentration) and emotional disturbances such as depression, irritability, emotional lability, and fatigue [37-40]. The consensus is that several autoimmune diseases do not normally present as single organ pathology. The final pathological state seen in disease may be determined by the initiation process/es that give/s rise to the condition; meaning that the quantitative amount, form and type of causal agent may have a lot to do with disease outcome. Concern is particularly expressed on exposures of pregnant women to a variety of xenobiotics. Following such exposures development of the fetus up to adolescence becomes critical with respect to health impact. Convincing and tangible reasons emphasize that females and children may have unique sensitivities to exposures to air pollution, solvents, pesticides, lead, mercury, and other

**Table 1:** Array of autoantibodies and organs affected

<i>Autoimmune Disease</i>	<i>Antigen/Organ affected</i>	<i>Abs/T-cell mediated</i>	<i>Specific Abs</i>	<i>HLA/MHC association*</i>	<i>F/M Ratio</i>	<i>RR*</i>
SLE	Systemic, kidney, skin, cns, cvs	*ANA-90% +	Anti-dsDNA	DR2	4/1	3
		*R F- 20% +	Anti Sm	DR3		3
Drug induced lupus	Organ or systemic	Ab related to drug-type	Anti-histone			
RA	Joints, vascular beds	ANA-20% + RF -90%+	Anti IgG (IgM=RF)	DR4	3/1	6
Wegener's granulomatosis	Blood vessels	Anti-neutrophil cytoplasmic proteins & lysosomes (Merck Manual 16 <sup>th</sup> edn.p1331)	C-ANCA		1/1.6 (PAN)	
Vasculitis	Small vessels, post capillary venules mostly (multiorgan); heterogenous		P-ANCA			
Scleroderma	Collagen: Skin/Distal extremities/Face/esophagus/kidney/lungs	ANA 95%	Anti-Scl-70 (insensitive)	DR1, DR3, DR5	3/1	
CREST			Anti-centromere			
Scleroderma (diffuse)	Internal Organs-heart, lung/kidneys	ADCC/T	Anti-PM-Scl -U3 nucleolar RNP		3/1	
Primary Biliary Cirrhosis	Liver-p74, E2 component of PDC	Ab/T	Anti-mitochondria (IgG)		High	
Celiac Disease	GIT/ Gluten (wheat)	Ab to gliadin fraction of gluten	Anti-gliadin	DR3 DQw2 B8		11.6 10
Goodpasture's syndrome	Type IV collagen of basement membrane  Kidney/Lung/Epithelial cell crescents	Ab GBM	Anti GBM	DR2		15.9
Pemphigus vulgaris	p130 Desmosomal cadherin + p85 plakoglobin	Ab-epithelial cells	Anti-epithelial cells	DR4 DRw6		
Hashimoto's thyroiditis (Hypo)	Thyroglobulin Thyroid ± (Schmidt's syndrome)	Ab	Anti-microsomal	DR5 DR3	8/1	3
Graves' Disease	Thyroid /TSHR	Ab	Anti-TSH/-TSHR/	B8 DR3	High	2.5 3.7

Multiple Sclerosis	MBP/PLP	2°Ab CNS/ T	Non-Specific	DR3 DR2	1/1	4 7
Myasthenia Gravis	ACHR	Ab	AntiA-CHR	DR3 B8	3/2	3 4
IDDM	Pancreatic $\beta$ cell ags, insulin	Ab/T	Anti-Insulin (Resistant state)	DR3 DR4 BfF1 Heterozygote DR3/DR4		4.8 3.6 15 33
Sjögren's syndrome	Lacrimal, salivary gland nucleolar ag.	ANA 70% +  *RF 75% +/T	Anti- Ro/SSA-60%+/ La/SSB-50% +/	B8 DR3 DRBw52	9/1	
Dermatomyositis & Polymyositis	Skin /skeletal muscle	Ab	Cytoplasmic rna Anti Jo-1, srp/T	DR3 DRw52	2/1	
Ankylosing spondylitis	Axial skeleton	Ab	$\uparrow$ IgA, APR	B27	1/9	121
Addison's disease	Microsomal proteins of Adrenal glands	Ab to ACTHR Adrenal cortex	Anti- ACTHR	B8 DR3 type II PGS DR4	1/1	7 10
Pernicious anemia	Gastric Parietal cell Ag Intrinsic factor Stomach	Ab toparietal cells	Anti-parietal cell autoantibodies			
Autoimmune Hemolytic Diseases	Red blood cells (Blood groups)	Ab/	Eg. Anti-D (rhesus)			
ITP	Platelet FcR Glycoprotein IIb-IIIa  Ib-IX complex	Abs following viral infections	IgG anti PI <sup>a</sup>		3/1	
Pemphigus	Skin	Ab	Ab to desmosomes			

Ab, antibody; Ag, antigen; Anti GBM, anti glomerular membrane; ANA, anti nuclear autoantibodies; ACHR, acetylcholine receptor; ACTHR adrenocorticotrophin hormone receptor; ADCC, antibody dependent cell cytotoxicity; APR, acute phase reaction; CREST, Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia; APR, Acute phase reactants; F/M, female/male; C-ANCA-cytoplasmic antineutrophil cytoplasmic antibodies: antiproteinase (Wegener's granulomatosis); ITP, Idiopathic thrombocytopenic purpura; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MBP/PLP, myelin basic protein/proteolipid protein; P-ANCA, perinuclear antimyeloperoxidase; (SLE, polyarthritis); PAN, polyarteritis nodosa; PDC-pyruvate dehydrogenase complex (E2 component = dihydrolipoamide acetyltransferase); PGS, polyglandular syndrome; NS, non-specific; Srp, signal recognition particle; RR\*, Relative Risk (MHC class II antigen related); TSHR, thyroid stimulating hormone receptor; T, T-cell or cellular responses; TSHR, thyroid stimulating hormone/receptor.

heavy metals.

Asthma, leukemia and learning disabilities are now believed to be on the increase among children. In recognition of such mounting problems the federal Executive order of 1997, "Protection of Children from Environmental Health Risks and Safety Risks" lays the responsibilities on health agencies to pay special attention to environmental risks to children. In response, the National Institute of Environmental Health Sciences, the U.S. Environmental Protection Agency, and National Center for Environmental Health, Centers for Disease Control and Prevention, have in unity fostered research programs that are intended to ultimately reduce the extent of adverse human health effects resulting from exposure to hazardous environmental agents. This program named under Centers for Children's Environmental Health and Disease Prevention Research have overall aims that call for the use of biologically based risk assessment models to propound hypotheses regarding factors that may heavily influence the health of children and women of childbearing age, and to utilize the information to prioritize research. This has led to the recent U.S.EPA restriction on 2 organopesticides, methyl parathion and azinphos-methyl (Guthion), commonly used for agricultural and domestic purposes [41]. This paper describes mechanisms of immunopathogenesis to self-molecules induced by cellular interactions that occur predominantly among immunocytes and molecules that participate in immune responses. Dysfunctions of T- and B-lymphocytes receptor and ligand interactions epidemiologically observed to lead to pathology in the immune system are described. To do so means identifying processes that keep self-tolerance in check. Processes leading to the dismantling of tolerance need to be elucidated. The genetics of autoimmune diseases are looked at from the point of view of abnormalities or deficiencies associated with the cluster of antigen determinants that participate in T-, B- cell surface receptors/ligand interactions.

Similar deficiencies in serum proteins that maintain homeostasis in the internal milieu have been identified. Pathways that lead to clinical autoimmune diseases are reviewed; and resistance to autoimmune induction in murine species is analyzed to delineate pathological agents that may be at play. Finally an attempt is made to screen known apoptotic sequences that may throw light on the induction of autoimmunopathogenesis leading to cognitive dysfunction involving learning and memory lapses. This approach becomes necessary because there is public health need to characterize pathways involved in the processes of autoimmunopathogenesis. Literature findings indicate that autoimmune-induction may involve different pathways in different races and therefore different mechanisms may explain why female blacks have higher prevalence of SLE. Individual susceptibility to and most significantly the nature of the etiological agent initiating the disease may be significant in the process and may dictate the ultimate disease type

and pathogenicity. This calls for a clear understanding of the routes of autoimmune inductions and to work out pharmacotherapeutic regimen to counteract effects on predisposed individuals of environmental exposures to xenobiotics: mercury, silver and cadmium and others of the pesticide series that can induce sensitization in humans.

### **Autoimmunity: Epidemiological Importance**

In the United States it has been difficult to evaluate chronic morbidity and disability due to autoimmune diseases. Retrospective analysis of data on 24 autoimmune diseases estimated on existing published cases using weighted prevalence rates from U.S. census dating from 1965 till 1995 projected an overall number of 8,511,845 (ie. 1 in 31) Americans to be suffering from one form and/or other of autoimmune diseases in 1996. Approximately, a total of 237,203 Americans develop autoimmune disease each year; a total of 6,722,573 women and 1,789,273 men were estimated to be afflicted with autoimmune disease. Rheumatoid arthritis, thyroiditis/hypothyroidism, uveitis and Graves' disease/hyperthyroidism and insulin dependent diabetes mellitus (IDDM) were found to have incidence rates of 23.7, 21.8, 18.9, 13.9, and 12.2 per 100,000 of the population, respectively. Computations showed that about 42,137 new cases of primary glomerulonephritis (3.6/100,000), multiple sclerosis (3.2/100,000), polymyositis/dermatomyositis (1.8/100,000) and systemic lupus erythematosus (SLE) (7.3/100,000) occurred in 1996. Based on these data it was projected that new cases for every 5 years are in the order of 1,186,015. Women are at 2.7 times greater risk than men to acquire autoimmune diseases (Table 1). The estimated trend in prevalent rate also showed increase over time [30]. Significantly table 1 demonstrates the relative risks associated with possession of certain MHC class II antigens although haplotype linkage with diseases is thought to be more definitive.

Individual autoimmune diseases have traditionally been studied as separate entities; but many may share common mechanisms of induction and/or pathogenesis either at the initiation or in the final pathways of immunopathogenesis. In this light, autoimmune disorders are important causes of morbidity and are actually predominantly implicated in diseases prevalent in American society; they do have indeed a greater impact on public health as to cost to the US health system in terms of diagnosis and health care delivery. Because most of these diseases are chronic and need intensive medical care they pose a considerable burden on the population. Studies involving animals and twin-SLE patients indicate that while genetic predisposition may be a prerequisite for the development of the disease, environmental and/or susceptibility genes are important for its full expression in the organs affected. Aging processes in particular influence the thymus; shrinking

with diminution of function is associated with age [42-44]. Approximately 90% of SLE patients have autoantibodies (ANA) and 20% of them also have rheumatoid factor (RF); on the other hand only 20% of rheumatoid arthritic (RA) patients have in their serum ANoAbs with more than 80% of them presenting with the diagnostic RF, an anti IgG autoantibody of mainly the IgM isotype. Similarly in SLE patients the presence of auto anti dsDNA and anti-Smith (anti-Sm) antibodies is diagnostic of the disease. The nature of spread of common autoantibodies such as the anti nuclear antibodies (ANA) among most autoimmune diseases reflects possible multipathways but highly probable associated common route at arriving at the final autoimmune disease.

### **Cells and Molecules in Autoimmune Reactions**

The main cellular vectors of immune responses are lymphocytes. Different functional types implement the tasks of memory, specificity and discrimination between 'self' and 'non-self'. Two main populations of lymphocytes, T and B cells are involved in the task of recognizing and responding to antigen. They have on their surface membrane a large number of recognition molecules for self-and non-self identifications. Each lymphocyte demonstrates a single specificity. On contact with antigen clonal expansion occurs to eliminate the offending agent and preservation of memory for future encounter with same or closely related epitope. Lymphocytes therefore are able to show a clonal diversity that imparts evolutionary advantage with the equally complex environment. Under normal circumstances the immune system is able to respond to such diverse foreign antigens and provides an effective protection against pathogens that cover much of phylogeny ranging from viruses and bacteria to complex multicellular parasites. Through random somatic recombination of genes that code for the antigen-binding domains of lymphocyte receptors diversity is generated to combat all forms of antigens. By the same token it is inevitable that membrane receptors that are potentially reactive with self-antigens can be generated.

Normally, cells of the immune system communicate among themselves and with other cells of the body by means of numerous types of signaling molecules that may be secreted by exocytosis, diffuse via the plasma membrane and released into the extracellular fluids, remain adhered to the cell surface and only influence cells that are in contact with the signaling cell, a process of autocrine and/or paracrine signaling. Immunocytes respond by means of specific receptors that are transmembrane proteins located on the surface of the target cell. The TCR is a transmembrane heterodimer consisting of either  $\alpha$  and  $\beta$  chains (for  $T_H$ ) cells, or  $\gamma$  and  $\delta$  chains, in association with cytotoxic ( $T_c$ ) lymphocytes. The TCR belongs to the tyrosine kinase-associated receptor class and it interacts with intracellular protein

tyrosine kinases as well as a number of adaptor proteins that relay kinase-dependent signals to appropriate effector pathways [2, 15, 45]. On the other hand T-lymphocytes affect cell-mediated immunity by means of complex association with major histocompatibility gene products. Interaction of T cells with specific membrane receptors results in cellular activation, division and differentiation. Cell division provides clonal expansion and an expansion of the selected cells specifically able to respond to the inducing antigen. This is the basis of immunological memory. Differentiation of activated lymphocytes into specific effector cells promotes the elimination of antigen.

Activation of T lymphocytes needs interactions of the multimeric TCR with a molecular complex detected on antigen presenting cells (APC), usually a macrophage [29]. Processed antigen in association with major histocompatibility complex (MHC) classes II molecule or I make up the molecular components. This Ag-MHC complex dictates the dual recognition of "self" and Ag by the TCR and are important for intercellular self/nonself discrimination. The MHC plays a critical role in the development of both humoral and cell-mediated immune responses. They present the antigenic peptides for recognition by the T cell repertoire. These receptors bind signaling molecules to initiate a response by the target immune cells. Signals from such association are relayed to the nucleus to result in gene expression. This is done by elaborate set of intracellular sequential signaling-proteins that become phosphorylated by protein kinases, dephosphorylated by protein phosphatases, or bind triphosphate nucleotides to produce proteins in an activated state. Downstream proteins may also be phosphorylated as part of the cascade process.

Two types of protein kinases are involved in the phosphorylation cascade:

- 1) serine/threonine kinases and
- 2) tyrosine kinases.

Phosphorylation of the corresponding proteins occurs via kinases associated with the respective amino acids as specified; some protein kinases are capable of carrying out both kinds of phosphorylations. Specific combinations of intracellular signals rather than single signal acting alone mediate cellular responses. The process can be likened to a neuron perception of various signals originating from several dendrites. Therefore the target cell needs to integrate the incoming informations that originate from separate signals in order to arrive at the appropriate response. That is to say the effects of different extracellular signals are incorporated to produce a net biological result. The complexity of the signal transduction processes and their integration to a common end result is exemplified by binding of antigen to the T cell receptor.

Complexity associated with TCR/ligand interaction is exemplified by nonobese diabetic (NOD) mice that

spontaneously develop insulinitis, a typical pathologic lesion for diabetes development. This autoimmune-associated disease evolves through several characteristic stages, commencing with peri-insulinitis and culminating in invasive and destructive insulinitis to overt diabetes. In NOD mice peri-insulinitis is routinely observed at 3-4 week of age, invading insulinitis at 8-10 wk and clinical diabetes is evident by 10-12 wk [46]. Mechanisms involved in the initiation and progress of insulinitis to overt diabetes are not well clarified. However general data suggest a role for negative T cell signaling pathways that occur via inhibitory receptors found in neonatal animals. Blocking of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) tended to induce disease by 3 wk of age in BD2.5/NOD TCR transgenic mice. However, late (after 17 days of age) blockade did not induce disease [47]. Early (at 2-3 wk of age) treatment of female NOD mice with anti-B7-1 mAb increased the diabetes incidence with reduction in incidence after CTLA-4Ig or anti-B7-2 mAb treatment. At 10 wk of age none of these reagents had any effect on disease progression [48].

The interpretation is that CD28/B7/CTLA-4 costimulatory pathway has important role in regulating disease induction but not progression early in T cell life or development. On the other hand the inhibitory costimulatory molecule programmed death-1 (PD-1) and its ligands, PD-L1 and PD-L2 have been shown to play significant role in regulating T cell activation and peripheral tolerance [49, 50]. PD-1 deficiency leads to lupus-like syndrome with glomerulonephritis (GN) and arthritis or fatal dilated cardiomyopathy, dependent upon genetic background of the animal [51, 52]. It has been shown for certain also that only the PD-1-PD-L1 pathway is incriminated in the regulation of both initiation and progression of autoimmune diabetes in NOD mice [53]. It is therefore not surprising that any errors of signaling from self-proteins can go so awry as to cause diseases in susceptible individuals.

Initiation of T cell activation occurs via binding of ligand to the TCR. This binding in turn provokes an intracellular signal that leads to a number of cellular responses. The process can be regulated at various points including at the receptor level, and at strategic points distal to the receptor complex that involves second messengers, specific genes activation based on the receptor type and subtypes that initiated the event. Antigen receptor ligation may stimulate a proliferative response, induce unresponsiveness (anergy) or induce apoptosis (Figure 2). This is possible as a result of the nature of the ligand/receptor interaction that has the physical capability to drive selectively recruited signals inside the lymphocyte, and thus defines the nature of the subsequent immune response. Similarly other agents such as xenobiotics may bind the receptor and cause abnormal reactions evidenced by experiments with murine species [54, 55].

It is also apparent that BCR-mediated signaling at different maturational stages lead to distinct biological

consequences. However, few literature experiments exist that define the biological linkage of  $Ig\alpha/Ig\beta/mIg$ -mediated signals to these unique biological responses. The ultimate fate of a B lymphocyte is the production of antibodies. The process is regulated also in a complex way. The link between the external and the inner environment is via the B-cell antigen receptor and its ability to transmit signals to direct the B cell along differentiation. The antigen receptor for B cell is a multimeric protein complex consisting of the membrane (mIg) forms of the selected immunoglobulin isotype and at least two further coat proteins  $Ig\alpha$  and  $Ig\beta$ .

The membrane immunoglobulin is considered the backbone of the receptor complex, and the cytoplasmic tails of the coat proteins are thought to be the signal-transducing component, connecting the antigen receptor to the tyrosine phosphorylation pathway in the cell. However additional co-receptors and signaling effectors is necessary for the regulation and transmission of exact signals, leading to a non-autoreactive, plasma cell or memory cell milieu. Some BCR signals like those initiated by Syk and the cytoplasmic tail of  $Ig\alpha$ , are necessary for both B-cell development and peripheral maturation. Btk and cytosolic tail of  $Ig\beta$ , are required only at selected checkpoints. These examples reflect underlying differences in how the BCR signals in development and in the periphery, to arrive at the cell fate. Clarification is still needed as to which aspects of the cytoplasmic tails of the immunoglobulin or the cytoplasmic tails of the coat proteins or both tails are required for transducing these signals.

#### *Maintenance of Self-tolerance*

Tolerance to body proteins or molecules is sustained by active elimination of self-displaying cells in the thymus. Naïve lymphocytes without high reactivity to self-peptides are positively selected within the thymus while those with high affinity-reacting peptides go through negative selection to be deleted from the medulla. Self-tolerance may be a meta-stable state whereby the immune system fails to react unfavorably against self-molecules, cells or tissues. While T lymphocytes are credited with the function of maintaining immunological tolerance [56, 57] interactions between APCs and lymphocytes play critical role also in self-tolerance. The thymus and the peripheral tissues are the central- and peripheral lymphoid-tolerance locales respectively. Positive and negative selection mechanisms occurring in the thymus are key to the induction of self-tolerant-cell repertoire, particularly early in life during maturation of the immune system. Peripheral tolerance mechanisms occur in tissues that are not immediately available to the thymus. These extrathymic mechanisms are thought to include deletion, brought about by apoptosis, anergy, ignorance and regulatory cells that contribute to keeping autoreactive lymphocytes under control [57]. Recent findings confirm

that a large number of molecules detected on APCs including many with tissue-restricted expression and/or 'peripheral' proteins are expressed in the thymus environs; these molecules afford means by which the thymus eliminate self epitopes [58, 59]. Strategic placement of tolerance inducing mechanism of such refinement is revealed by studies with *Ins2* and *Ins1* knockout mice. These transgenic mice lack expression of insulin epitopes in the thymus and therefore cannot clear insulin molecules that gain entrance into the thymus environ from the pancreas [60]; thus leading to termination of tolerance to insulin with subsequent induction of type 1 diabetes autoimmune disease.

Majority of B-lymphocytes do require cooperation from a population of T helper (Th) cells in mounting an immune response against some types of antigens (Figures 1 and 2). Any compromise in the functional activities or lack of Th assistance therefore leads to depletion of specific antibodies to fight an invading foreign body. Normal individuals have low levels of auto-reactive B-lymphocytes in circulation, and in fact autoantibodies are present in the serum [61] but low enough to be of no concern. Some T lymphocytes bearing lower affinity 'self' MHC-peptide complexes are not eliminated in the thymus and do enter peripheral circulation. These potential self-reacting cells are, however held in check by clonal anergy (long-lived unresponsiveness) wherein they are incapacitated in mounting any effective immune response against self-molecules. Clonal anergy also prevents the precipitation of autoimmune responses by T lymphocytes reactive with 'self' antigens that are not encountered in the course of programming and selection processes [44, 56]. In fact it is only within the last decade with advancement in flow cytometric techniques that the immune cells are being categorized functionally. CD4<sup>+</sup> lymphocyte population that constitutively bear the interleukin-2  $\alpha$ -chain CD25 and commonly referred to as T regulatory (Treg or CD4<sup>+</sup>CD25<sup>+</sup>) cells, have been noted to be responsible for peripheral resistance [62, 63]. These lymphocytes express the typical  $\alpha\beta$  TCR and arise in the course of normal T-cell development as well as during the peripheral T-lymphocyte response to antigens under the control of cytokines, and costimulatory milieu. These lymphocyte subsets have been found to be central in controlling autoimmunity, tumour immunity and transplantation tolerance in several murine and in human's circulating blood lymphocytes, cord blood, tonsils, thymus and even in normal lymph nodes where they express peculiar phenotypic profile and TCRV $\beta$  repertoire [62-65].

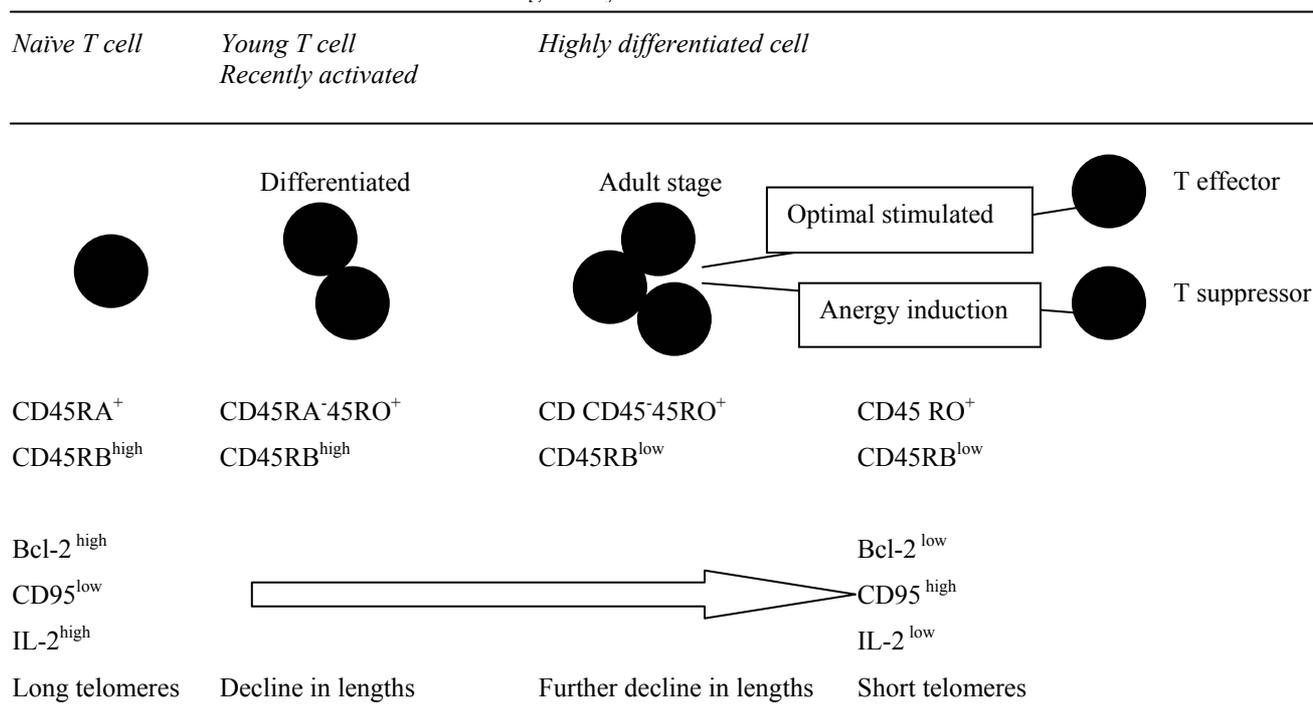
Thymic CD25<sup>+</sup>CD4<sup>+</sup> T cells suppress other CD25<sup>-</sup> autoimmune-inducing T cells and provide one of the important means for generating self-tolerance. Treg T cells are anergic; they were once thought to be incapable of proliferating in response to mitogenic antibodies to the T cell receptor (TCR) complex. But recent data indicate that they do proliferate even when no cytokines (IL-2) are detected in cell culture environment as well as

*in vivo* when activated by antigen-loaded dendritic cells (DC); more so with mature dendritic cells [62, 63]. Growth of CD25<sup>+</sup>CD4<sup>+</sup> T cells was halted by day 5 irrespective of the presence or absence of exogenously introduced IL-2, while CD25<sup>-</sup>CD4<sup>+</sup> T cells continued to proliferate: growth of CD25<sup>+</sup>CD4<sup>+</sup> T cells required contact with dendritic-T cells and this proximity was partially influenced by small amounts of IL-2 synthesized by T cells and B7 costimulation by the DCs. The CD25<sup>+</sup>CD4<sup>+</sup> T cells retained their surface characteristics *in vivo* and were found to actively suppress CD25<sup>-</sup>CD4<sup>+</sup> T cells in the absence of specific antigen but in the environment of exogenously introduced IL-2. Mature antigen processing DCs and those in a steady state environment induced proliferation of adoptively transferred CD25<sup>+</sup>CD4<sup>+</sup> T cells [63]. This finding shows that the ability for the environmental condition to induce the CD25<sup>+</sup>CD4<sup>+</sup> T cells provides another means for the DCs to regulate immune responses. This discovery has been reinforced with the finding that regulatory Treg T cells naturally impose a negative feedback mechanism on Th1-type responses that are induced in *in vivo* tests with DCs [59, 60]. Treg cells act as naturally anergic cells capable of suppressing the activation/proliferation of other lymphocytes, both CD4<sup>+</sup> and CD8<sup>+</sup> to CD3/TCR-mediated stimulation. They are also prone to apoptosis. Therefore Treg do modulate immune responses to both self and foreign antigens and play important role in maintenance of self-tolerance.

In both murine and in adult humans the general observation is that T reg cells though are anergic are also highly differentiated. This has led to the belief that a subpopulation of Treg (CD25<sup>+</sup>CD4<sup>+</sup>) cells probably arise in the periphery as a result of induced anergy in CD4<sup>+</sup> T cells that are at their terminal differentiation [65]. The basis for such opinion stems from noted acquisition of effector functions when T cells proliferate upon repeated stimulation with antigen over time. Under such circumstances a change in expression of surface receptors are observed, including dynamic changes in cytokine, homing, adhesion, and signaling receptors [66].

Further support for induced anergy from repeated stimulation of Treg cells come from the finding that naïve or recently stimulated T cells express high levels of CD45RB while highly differentiated CD4 T cells express low levels of this molecule [67]. T reg cells in mice demonstrate the phenotype CD45RB<sup>low</sup> and in humans CD45RO<sup>+</sup> CD45RB<sup>low</sup> phenotypes are detected; meaning that they usually experience many cell divisions and therefore undergo continuous re-activation towards growth arrest, a process known as replicative senescence after a finite number of cell divisions; table 2. Replicative senescence is thought to be a strategy used by growing cells to guard against potential malignant expansions of cells [68]. This protection against malignancy operates to result in gradual shortening of

**Table 2:** Tolerance Induction in CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells.



Generation of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in the periphery of immune system– naïve CD4<sup>+</sup> T cells become primed upon encounter with antigen on APCs, lose CD45RA and acquire CD45RO expression. These cells remain CD45RB<sup>high</sup> during this process and retain the capacity to secrete large amounts of IL-2. They still express Bcl-2 and have long telomeres. Repeated stimulation of the primed cells by frequently encountered antigen leads to generation of highly differentiated CD4<sup>+</sup>CD45RO<sup>+</sup>CD45RB<sup>low</sup> memory/effector T cells. These highly differentiated T cells have short telomeres and are prone to apoptosis, as they express low levels of Bcl-2. They also have a profoundly diminished ability to secrete IL-2, which makes them susceptible to anergy induction. These cells can either remain as effector T cells, or they can become anergised and acquire suppressive capacity [3, 68, 69, 151-153].

telomeres as repeated cell divisions continue. Telomeres are repeating hexameric nucleotide units at the ends of chromosomes. These units are known to shorten by about 50 base pairs with each cell division [69, 70]. Aged T cells may therefore be useful to offer a plausible mechanism by which high concentrations of antigen may influence the development of specific regulatory cells, a concept that is well in conformity with the idea of high-zone tolerance.

*Maintenance of Self-tolerance: Role of CD95 and Ligand*

In humans as well as in murine species, CD95 (Fas/Apo-1) and its ligand (CD95L) have been demonstrated to play significant roles in the regulation of lymphocyte populations [71, 72]. Mutation of the normal gene product for either of the molecules results in lymphoproliferative disorders and increased susceptibility to autoimmune syndromes. Murine strains with *lpr/lpr* homozygous genes contain mutations in the CD95 gene that render the products nonfunctional. Similar findings have been observed in the *gld* mice

that also have deficient CD95L, indicating that this receptor/ligand interaction is very important and critical in many T cell functions such as thymocyte development and apoptosis of mature peripheral T cells [71-76].

Many laboratories have confirmed that both CD95 and its ligand play a critical role in immune privileged sites (Figure 2). Certain tissues such as the eye and the testes are protected from lymphocyte invasion than other organs. Cells within both the eye and the testes express high numbers of CD95 ligand [77-79]. Stimulated lymphocytes that enter these sites are inflammatory and express high levels of CD95. The interaction of the CD95 on the invading lymphocytes and the CD95L on these tissues leads to apoptosis of the former. This interaction thus affords a protection from immune-mediated destruction. The CD95/CD95L interaction has been shown to implement systemic immune tolerance against inciting antigens [78, 79].

More recent findings indicate that the Fas ligand in addition to inducing death and acting as a costimulator of peripheral T cell activation can also function as an accessory molecule in positive selection for thymocytes [78]. Immune privilege therefore is an active process

involving elimination of antigen-specific T cells in a way that prevents future response to the specific antigen. It is when immunological tolerance is broken that autoimmune responses and autoimmune diseases ensue.

#### *Termination of Tolerance*

Absence or loss of self-tolerance exposes one to the induction of autoimmune responses leading to the autoimmune state that can end up in cellular and tissue damages. Tolerance can be broken or bypassed by a variety of mechanisms. Release of previous cryptic (hidden/latent) antigens unavailable to thymic programming is one means of overcoming existing tolerance. Traumatic damage to tissues can initiate this process. Cells that do not normally express MHC class II products may carry self-antigens that are not detected by T lymphocytes that require the class II molecules (CD4<sup>+</sup> Th cells) for self-identification. Certain cytokines like interferon- $\gamma$  (IFN- $\gamma$ ) can stimulate cells that do not normally express MHC class II to acquire these molecules. This process can result in the stimulation or augmentation of inappropriate immune reactions against self-antigens. Molecular 'mimicry', a process whereby self-molecules that share homologous determinants with invading organisms react to antibodies formed in the process of counteracting attacks from the invader. An example is infection with *Streptococcus* that leads on to rheumatic fever; membranes from  $\beta$ -hemolytic bacterium cross-react with cardiac myosin induce antibodies against cardiac muscle [80]. Modification of self-peptides or MHC determinants by chemicals is another route of breaking self-tolerance. In this arena are found metals that can induce autoimmune state secondary to the induction of new high affinity sites for MHC determinants on self-peptides. Hapten-specific T lymphocytes can help B-lymphocytes to identify the haptenated self-protein and therefore initiate an immune response against own tissue. Polyclonal activation directly or indirectly stimulates B lymphocyte populations to elaborate polyclonal antibodies, auto reactive antibodies included [81, 82]. Non-cytotoxic low concentrations of some xenobiotics such as mercuric chloride (1-10  $\mu$ M) can attenuate CD95-mediated apoptotic cell death culminating in accumulation of self-antigens in the microenvironment that can lead to breaking of tolerance threshold [54, 83].

Relatively recent data implicate complement proteins as playing significant roles in either the maintenance or acting to tip the balance towards autoimmune induction. In this arena are observed development of SLE as a result of complement deficiency syndromes. One of the invariant features of SLE disease is a severe photosensitive skin rash frequently accompanied by high titers of anti-Ro (SSA) antibody [84]. These subgroups of patients are usually younger in age with no female predominance, probably indicating no other genetic involvement. These patients also have intact alternative

pathway of complement system that can clear immune complexes if need be. Also patients with C2 deficiencies do develop lupus despite that they have intact classical pathway to clear complexes through C4b coating. These arguments go against the hypotheses that impaired clearance of antigen-antibody complexes leads to development of SLE in patients with complement deficiency. However, it is observed that the skin of this SLE subgroup of patients may function both as the primary target of the immune system as well as the site at which tolerance is initially broken. Keratinocytes that undergo apoptosis through exposure to ultraviolet light generate discrete subcellular surface blebs that contain nuclear or cytoplasmic constituents exclusively, many of which are targets for autoantibody synthesis [85, 86].

High concentrations of autoantigens and associated specific apoptotic proteases have been isolated from these blebs. The potential immunogenicity of apoptotic cells is supported by in vivo studies involving normal mice that are injected with syngeneic thymocytes. These mice when exposed to gamma irradiation develop lupus-like symptoms including autoantibody production and IgG deposition in the glomeruli [86]. Such studies have proved unambiguously that self antigens are concentrated and clustered in blebs of apoptotic keratinocytes suggesting that C1q play critical role for proper recognition, clearance, and processing of self-antigen localized within surface blebs generated by apoptotic cells.

#### **Genetic Determinants of Autoimmune Diseases**

Many genes are involved in lupus-predisposition [87-98]. Mutations occurring in these genes result in either enhancement or reduction to lupus-like diseases. The MHC and non-MHC genes, as well as susceptibility to spontaneous lupus influence the predisposition to autoimmune diseases. Systemic lupus erythematosus (SLE) presents as chronic autoimmune disease with highly heterogenous clinical features of variable pathogenicity. Epidemiological data suggest a strong genetic component for susceptibility to SLE [42, 99-105], and multiple genes involving those that affect immune complex deposition do play roles in pathogenesis as well [103-105]. Generally subjects carrying certain HLA alleles have a higher risk of specific autoimmune diseases as compared to controls without these alleles. The 8.1 ancestral haplotype (AH) is an example found among Caucasians who type for HLA-B8, DR3. The allele is part of the AH8.1HLA-A1, Cw7, B8, TNFAB\*a2b3, TNFN\*S, C2\*C, Bf\*s, 4A\*Q0, C4B\*1, DRB1\*0301, DRB3\*0101, DQA1\*0501, DQB1\*0201 [106]. Because of their close linkage on the same chromosome they are inherited together, hence are referred to as ancestral when they define highly conserved haplotypes that appear to originate from a common ancestor. This haplotype is associated with a

wide range of immunopathological diseases. Comparative oligonucleotide microarray screening and other tests and analyses of samples from SLE patients in active disease phase versus control patients reveal the presence of at least 20 SLE susceptible loci [107]. These loci have been reported to have a minimum of 61 genes with global spread that are implicated in SLE disease susceptibility. These findings reinforce that SLE is inherited as a polygenic disorder with contributions from multiple genes (each with its own degree) to pathogenesis. Mendelian inheritance is also suggestive [107-109]. The level of gene expressions in the test patients was found to be significantly ( $p < 0.05$ ) increased 2-fold above the level of those of the control samples. Out of the 61 genes isolated and that differed significantly from the controls, 24 were upregulated, and 37 were downregulated. Several related groups of genes were observed and most of them function in various different biochemical pathways. Among the differentially expressed genes, interferon paths involving *IFN- $\omega$* , IFIT1 (interferon-induced protein 56), IFIT2 (interferon-induced protein 54), IFIT4 (interferon-induced protein with tetratricopeptide repeats 4), OAS1 (2' 5'-oligoadenylate synthetase1), OAS2 (2' 5'-oligoadenylate synthetase2) OASL (2' 5'-oligoadenylate synthetase-like), and *Ly6E* [(lymphocyte antigen 6 complex, locus E) (TSA-1/Sca-2)] were elevated in expression in SLE patients. In the same patients the T-cell receptor (TCRs) pathways, TCR  $\alpha$ , TCR  $\delta$ , and 2' 5'-oligoadenylate synthetase were decreased in expression. *IFN- $\omega$*  and *Ly6E* (TSA-1/Sca-2) were thought to play significant roles in the mechanism of SLE pathogenesis [108].

Investigations with *NZM2410* strain of mice also show that the *Sle1* is one of potent loci that trigger the formation of IgG anti-histone/DNA antibodies when expressed on the B6 background as a congenic interval [87, 91]. The *B6.lpr* lines by contrast exhibit distinctly different cellular and serological phenotypes. Both strains do not normally exhibit pathogenic autoantibodies, or are subjected to lupus nephritis. Data indicate that the epistatic interaction of *Sle1* particularly the *Sle1/Sle1* with *FAS<sup>lpr</sup>* leads to massive lymphosplenomegaly (with increased numbers of activated CD4 T cells, CD4<sup>+</sup>CD8<sup>-</sup> double negative (DN) T cells, and *B1a* cells), elevated IgG and IgM antinuclear levels (including anti-ssDNA, anti-dsDNA, and anti-histone/DNA), and antiglomerular autoantibodies. There was histological and clinical evidence of glomerulonephritis (GN) and significant percentages of mice mortalities by 5-6 months of age. The *FAS<sup>lpr</sup>* was found to be recessive in function while the *Sle1* exhibited gene dosage effect; signifying that *Sle1* and *FAS<sup>lpr</sup>* probably act in alternate pathways culminating in hyperproliferation of lymphocytes that leads to autoimmune disease [87]. These susceptibility genes, in addition to the NZB trait localize to the *Hmr1* locus. *Sle1a-c* from the NZM/Aeg2410 and NZW backgrounds

and *Bxs3* from the BXS strain are other predisposing loci in this region [93-96]. Highly lupus-susceptible lupus murine strains except MRL- *FAS<sup>lpr</sup>* have been found to have loci that overlap with the *Hmr1* interval. Genes that are involved with predisposition to the development of lupus include *gld* [72, 97] *Fasl* [71-79, 97], serum amyloid P-component (*Sap*) [98, 110-115], *Fcgr2b* (*Fc $\gamma$ RIIB*) [116], *Cr2* (*CD21/CD351*) [117, 118], and *Ptprc* (*CD45*) [119], whereas deficiency of *Fcrlg* (*Fc $\gamma$ -chain*) [116, 120] results in resistance to autoimmunity. Localized genes in this region may explain some of the linkage of systemic lupus erythematosus traits to homologous areas on human chromosome 1 in various populations [87, 102, 105, 121, 122].

Increased interferon alpha (*IFN- $\alpha$* ) is one of the first cytokine abnormalities characterized in SLE patients [109]. Later *IFN- $\omega$* , formerly called *IFN- $\alpha$  II* because of its 60-70% share of sequence homology [109, 124] with *IFN- $\alpha$*  was detected in the sera of SLE patients and is believed to be an important *IFN* product in the sera of SLE patients [124-129]. In patients with spontaneously induced SLE high levels of *IFN- $\alpha$* -inducible proteins (eg *OAS1*, *OAS2*, and *OASL*) and other proteins have been measured [108, 128-130]. Clinical observations indicate that when non-SLE afflicted patients are treated for other diseases with *IFN- $\alpha$*  for long-term period they can induce formation of anti-nuclear antibodies against native DNA that specify the SLE disease [130-132]; conditions found also in *HgCl<sub>2</sub>*-treated rats [53]. Besides its antiviral activity, *IFN- $\alpha$*  is also associated with several immunomodulatory functions. It can stimulate immunoglobulin (Ig) synthesis in peripheral blood mononuclear cells (PBMC) *in vitro* [130], maintains T cells alive [132], inhibits B cell receptor-mediated apoptosis [133], and is able to induce dendritic cell differentiation in SLE patients [134, 135]. For these reasons *IFN- $\alpha$*  has been observed to play a very significant role in the initial immunopathogenesis in SLE groups [136, 137].

Although the *Ly-6* families of molecules are yet to be fully assigned biologic roles evidence so far associate them with intercellular adhesion and signaling functions. Among the *Ly-6* family is *Ly6E*, named as *TSA-1* (thymic shared antigen-1)/*Sca-2* (stem cell antigen-2). All immature thymocyte subpopulations of CD3<sup>+</sup>4<sup>+</sup>8<sup>-</sup>, CD3<sup>+</sup>4<sup>+</sup>8<sup>+</sup>, CD3<sup>-</sup>4<sup>+</sup>8<sup>+</sup> and CD3<sup>-</sup>4<sup>+</sup>8<sup>-</sup> display this *TSA-1* molecule. However peripheral T cells and early migrating CD3<sup>+</sup>4<sup>+</sup>8<sup>-</sup> and CD3<sup>+</sup>4<sup>+</sup>8<sup>+</sup> thymocytes do not display any *TSA-1*. For this reason *TSA-1* has been used as a marker to differentiate thymocyte subsets that are immature. B-lymphocytes in the periphery do possess *TSA-1* [137-139] and there is an inverse relationship between *TSA-1* and *CD3* expression on thymocytes confirmed with oligonucleotide microarray technique [107, 139]. Han and his group detected lower expression of TCRs pathways (TCR $\alpha$ , TCR  $\delta$ , and Zap-70 associated protein kinase 70kDa) than control levels;

this confirms many literature data indicating TCR/CD3 defects in SLE patients [107, 137, 139-142]. The inverse relationship between TSA-1 and CD3 expression on thymocytes may be an indication or diagnostic of abnormally activated lymphocytes in SLE patients. Expression of surface Ly-6 antigens on T cells *in vivo* and *in vitro* is highly increased by IFN- $\alpha/\beta$  [142]. Quantitative numbers of TSA-1 mRNA in human PBMC are also dramatically increased by IFN- $\alpha$  and slightly by IFN- $\gamma$  [107, 108]. Protection is afforded by anti-TCR/CD3-induced apoptosis in immature thymocytes by a signal transduced via TSA-1/Sca-2, and modulation of TCR-mediated signaling route by TSA-1/Sca-2 [143]. These data infer that TSA-1 antigens represent important alternative pathway for T-cell activation in IFN-mediated immunomodulation found prominent in SLE patients; and is a pathway used in SLE-prone patients to initiate the autoimmune state. Similar multichannel pathways are also emerging from studies with patients suffering from rheumatoid arthritis (RA) [143, 144].

RA synovial tissues of varying disease severity or inflammation have recently been derived from samples of rheumatoid arthritic patients. Based on stage of tissue pathology these patients were classified into two major types: RA-I and RA-II relying on hierarchical clustering of gene expressions. A striking variation between the groups was the level of expression of immune-related genes. The RA-I tissues showed elevated inflammatory gene expressions. Highly expressed genes point to specific activity of B and T cells and molecules that constitute the TCR signaling complex such as CD3  $\delta$ , T-cell-R  $\alpha$ ,  $-\beta$  and  $-\delta$  locus- and *lck* products that were detected in the RA-I groups [143]. Also noted were genes that expressed IL-2R common  $\gamma$ -chain, the IL-7R, *stat1* and the cytokine/*stat*-activation pathway-induced gene (*SS13*). Ongoing immune responses in the patient samples were detected by the presence of HLA class II encoding genes, IFN  $\gamma$ -induced genes such as *IFI30* and *IRF1* were also described. High degrees of T- and B-cell stimulations that expressed genes regulating lymph node development (*DOCK2*) and *BlyS* [144-146] were present. B lymphocyte activators (*TNFSF13b*) were identified within the RA-Ia subgroup. Similar gene expressions were found in the RA-Ib subgroup. In addition, the RA-Ib subgroups demonstrated signs of the classical pathway of complement activation: *C1Q  $\alpha$* , *C1Q  $\beta$* , *C2* and *C1R* and an inhibitor of complement lysis, clustering was active in this subgroup.

One interesting conclusion from this work is that no clinical variables could be utilized to differentiate age of disease onset or disease duration. Laboratory measurements or medication between the groups of patients analyzed did not yield any clear demarcations. This points out the difficulties involved in differentiating pathological processes that are active in late stage RA that may not be easily recognized in clinical diagnostic procedures. Susceptibility to autoimmune disease may therefore be operating via multiple pathways and stages

or checkpoints as clinically seen in SLE and in RA patients [87, 142-149]. In genetically predisposed patients, the generation of autoantibodies may vary between genetically susceptible and non-genetically prone patients; ethnicity may play a role as well in the pathway of autoimmune induction. Treatment schedules need to vary also. Elucidating and understanding the complex mechanisms leading to genesis of systemically induced autoimmune states seen in the fibrotic processes in scleroderma and the development of SLE and RA disease in man will be a great step in laying the foundation for pharmacotherapeutic managements of each and every case of immune dysfunctions associated with any ailment in various ethnic groups.

#### *Cytokines and Receptors Regulate Pathogenicity in RA Autoimmunity*

Findings from genetic studies usually yield important information to assist clinical managements of patients with autoimmune diseases. Literature review on rheumatoid arthritis as an example indicates that mechanisms are at play in the induction of RA in the synovium. The use of animal models of inflammatory arthritis and data from patients with rheumatoid arthritis reveal that several cytokines are produced in the course of the disease; see Table 3, Figure 4. Cytokines are considered to be factors that mediate communications between cells, and are involved in attracting inflammatory and immune cells into the joints where these cells emit products that are damaging to the tissues. Cytokines interact specifically with receptors on cell surfaces; stimulate pathways of signal transduction leading to high or low transcription. Two-signal transduction pathways that may be considered important in the rheumatoid synovium are the AP-1 and the NF- $\kappa$ B pathways [52, 53, 150]. The later seems to be particularly important in chronic inflammatory diseases, both in mediating IL-1 and TNF $\alpha$  production as well as in mediating their effects on target cells after binding to cell surface receptors. Stimulating these AP-1s and the NF- $\kappa$ B signal transduction pathways causes release of collagenases and other enzymes, other pro-inflammatory molecules, and more cytokines. Cytokines are grouped according to function in the rheumatoid disease process ranging from hemopoietic, growth and differentiation, immunoregulatory, pro-inflammatory, anti-inflammatory, to chemotactic factors. Any cytokine however, may function under more than one of these categories. Cytokine receptors are classified on the basis of structural similarities and usage of common molecules for signal transduction. Studies on the role of cytokine in the generation, maintenance, and dissolution of disease have been utilized to arrive at possible therapeutic schedules based on quantitative, qualitative cytokine factors in operation at any one time. Cytokines in the rheumatoid synovium operate in a network of overlapping, synergistic, antagonistic, and inhibitory

activities. Therefore the net biologic response depends on the balance between multiple types of cytokines present; meaning that positive and negative response to cytokines can be viewed in terms of severity or recovery from disease.

Three types of cytokine patterns have been assigned to chronic rheumatoid synovium, correlating with different histologic views [151, 152]: specimens with diffuse lymphoid infiltrates with no other arrangements, specimens that exhibit lymphoid follicles with germinal center (GC) formation, and specimen that show granulomatous synovitis. Those specimens with diffuse lymphoid infiltrates showing no further arrangements have been noted to have low levels of mRNA for IFN $\gamma$ , IL-4, IL-10, and TNF $\alpha$  (Th0 cytokine pattern); specimens with lymphoid follicles and GC formation contained mRNA for IFN $\gamma$  and IL-10 but not for IL-4 (Th1 cytokine pattern). High levels of mRNA secreting IFN $\gamma$ , IL-4, IL-1 $\beta$ , and TNF $\alpha$  (mixed Th1/Th2 cytokine pattern) characterized granulomatous synovitis specimens. These specimens therefore demonstrated heterogenous cytokine patterns that indicated pathophysiologic mechanisms that can vary among persons. Cytokines also drive immunocyte differentiation in the rheumatoid synovium with T cells carrying characteristics of mature memory cell (CD45RO<sup>+</sup>). Interleukin 15 instead of IL-2 has been stimulation. This cytokine produced by macrophages, observed to be the cytokine causing the T cells

fibroblasts, and endothelial cells is assigned many functional attributes, some similar to that of IL-2 and including binding to a receptor with a unique  $\alpha$  chain but uses the  $\beta$  and  $\gamma$  chains of the IL-2 receptor (IL-2R) to transduce signals in target cells [152, 153]. IL-15 is chemotactic for CD45RO<sup>+</sup> T cells and activates these cells to produce TNF $\alpha$  in the synovium. Table 3 shows classification of cytokines.

Though many of the cytokines associated with RA are known the mechanism and the cytokine that initiate the disease is still evasive. Both antigen-specific and non-antigen specific mechanisms are postulated to be involved. Activated T cells with the HLA-DR4 MHC epitope is thought to initiate the process and through cross-reaction with self-antigens, leads on to stimulation of macrophages via release of cytokines such as IFN $\gamma$  and by direct cell-to-cell contact. On the other hand the non-antigenic mode of initiation of RA is thought to arise through the episodic release of TNF $\alpha$  and GM-CSF from synovial macrophages and fibroblasts that may have been traumatized, infected or through immune-complex deposition [153]. The effects of cytokines in the development of disease may be local or systemic. The acute phase response involves increased levels of proteins of the complement system, coagulation and fibrinolytic systems, antiproteases, transport proteins, and other proteins that participate in the inflammatory response, such as the cytokine IL-1Ra. IL-6 and other

**Table 3.** Classification of cytokines

<i>Family</i>	<i>Cytokine nomenclature</i>
Hematopoietic	SGF, IL-3, TPO, EPO, GM-CSF, G-CSF, M-CSF
Growth and differentiation	PDGF, EGF, FGF, IGF, TGF $\beta$ , VEGF
Immunoregulatory	TGF $\beta$ , IFN $\gamma$ , IL-2, 4, 5, 7, 9-18
Pro-inflammatory	IL-1 $\alpha$ , IL-1 $\beta$ , TNF $\alpha$ , LT, IL-6, LIF, IL-17
Anti-inflammatory	IL-1Ra, IL-4, IL-10, IL-13
Chemotactic	IL-8, MIP-1 $\alpha$ , MIP-1 $\beta$ , MCP-1, RANTES

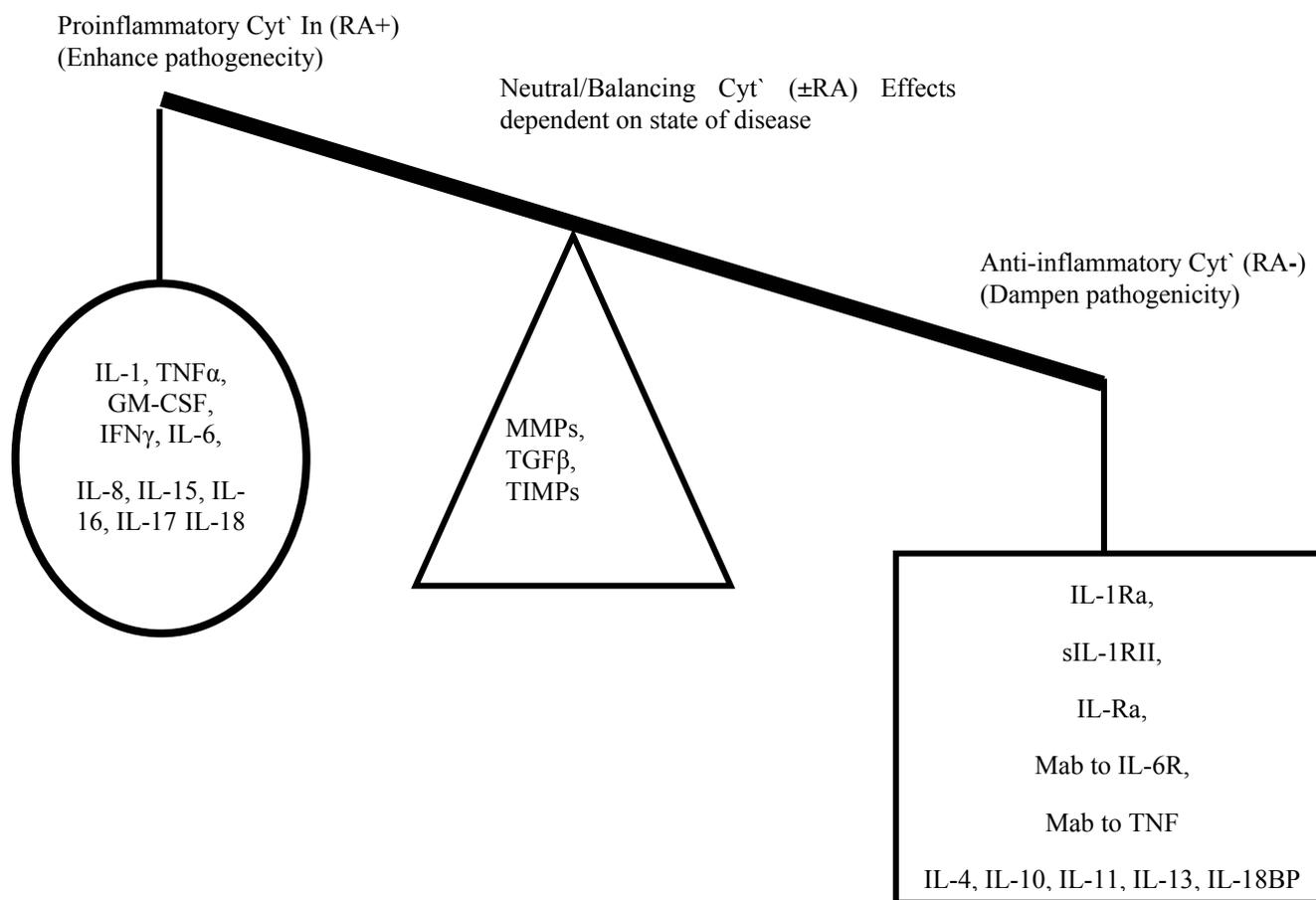
EGF, epidermal growth factor; EPO, erythropoietin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IGF, insulin-like growth factor; IL-interleukin; IL-1Ra, interleukin-1 receptor antagonist; LIF, leukemia inhibitory factor; LT, lymphotoxin; MCP, monocyte chemotactic protein; M-CSF, macrophage colony-stimulating factor; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated upon activation, normal T cell expressed and secreted; SCF, stem cell factor; TGF, transforming growth factor; TNF, tumor necrosis factor; TPO, thrombopoietin; VEGF, vascular endothelial growth factor. [151].

cytokines commence synthesis of these proteins by stimulating hepatocytes in the course of either acute, chronic as well as malignancies and infections. Because most of the functions of these cytokines are now familiar their clinical uses together with monoclonal antibody to receptors/cytokines are becoming more and more available for therapy.

Quantitative and qualitative analysis of cytokines in synovium of RA patients are pathophysiologically described as self-regulating. By the combined actions of anti-inflammatory, antagonism of other cytokines and/or probable use of naturally occurring antibodies to cytokines, (Figure 4), an attempt is made to provide natural body products to combat disease severity; (see also table 3). An imbalance between these cytokine networks is believed to cause disease either from excess of pro-inflammatory ones or from inadequate availability of natural anti-inflammatory cytokines. Currently known major cytokines that possess anti-inflammatory effects include IL-4, IL-10, IL-13, TGF  $\beta$ , and IL-1Ra; IL-10 and IL-1Ra are predominant in rheumatoid synovium [154]. Interleukin 10 may inhibit IL-1 and TNF $\alpha$

production and cause elevation of IL-1Ra. TGF  $\beta$  may display both pro- and anti-inflammatory effects dependent on the species. In RA humans TGF  $\beta$  may suppress T cell functions, inhibit fibroblast growth and production of tissue-damaging enzymes, and also increase the production of specific inhibitors of these enzymes. Experimental evidence indicate that IL-1Ra, a structural variant of IL-1 that binds to IL-1 receptors on target tissues but fails to stimulate any intracellular responses can function as a specific inhibitor of the effects of IL-1 [155]. Transgenic mice lacking IL-1a are found to suffer collagen-induced arthritis, a model of human RA [156].

The presence of high tissue levels of IL-1Ra may therefore afford a protection against spontaneous development of inflammatory disease of the joints or vessel walls in mice. Further investigations on IL-1Ra may afford some relief in humans with RA. On the same theme the use of antibodies to TNF $\alpha$  seems to significantly inhibit cell trafficking into the joints secondary to decreased expression of adhesion molecules on endothelial cells. Currently, avenues



**Figure 4:** Equilibrations in Rheumatoid Synovitis in RA - Restoration of equilibrium in rheumatoid synovitis. The balance is shifted to the right with out of size rectangle, indicating that the anti-inflammatory cytokines are dominant. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IL-1Ra, interleukin -1 receptor antagonist; TGF, transforming growth factor; TNF, tumor necrosis factor. Cyt' - cytokine [153-155].

for therapy for RA patients are designed to restore balance between these positive and negatively disease-inducing cytokines including the utilization of monoclonal antibodies to TNF $\alpha$ , soluble TNF $\alpha$  receptors, and IL-1Ra.

### Pathways Leading to Predisposition or Resistance to Autoimmunity

Original studies with animals laid down the foundation for subsequent flood of investigations of how some individuals are predisposed to developing autoimmune state. Findings from such studies showed that:

1. The strongest MHC haplotype association with autoimmunity involves the class II *DQB* where high titers of IgG anti DNA are measured in lupus nephritis. Certain haplotype combinations such as *DQB1\*0201, 0602* and *0302* with *DR2* or *DR3* [117, 118, 120] are highly associated with autoimmune induction. Antibodies raised to Ro/La (SS-A/SS-B) antigens for example are associated with dermatitis of subacute cutaneous lupus and with certain *DQA* and *DQB* genes inherited with *DR3* and occasional *DR-2* [157]; Table 1.
2. Deficiencies of proteins important for physiological maintenance of homeostasis lead on to immune dysfunction of autoimmune nature. These include serum amyloid P component (SAP), C-reactive proteins (CRP), general lymphoproliferative disease (*gld*) gene products, Fas and ligand (CD95/FasL) and CD45, Fc gamma IIb (Fc $\gamma$ RIIB), complement receptor 2 (Cr2), Ptpcr, TSA $\alpha$  (adaptor protein) and the inhibitory costimulatory molecule programmed death-1 (PD-1) and its ligands, PD-L1 and PD-L2 detected in NOD mice [4 9-53, 98-115].

### Serum Amyloid P Component and C - reactive protein Involvement in Autoimmunity

Mice with serum amyloid P (SAP) component deficiency (SAP $^{-/-}$ ) develop and breed normally; but they spontaneously produce high titers of antinuclear antibodies against chromatin, DNA and histones. Enhanced production of these molecules occurs with increasing age from 3-months [98-115] and results in severe GN similar to findings in patients with SLE disease. SAP is a highly conserved plasma protein universally present in amyloid deposits [110-112]. Under physiological conditions SAP, through calcium binds avidly to DNA and chromatin [114]. Strong binding of SAP displaces H1 type histones and thus solubilizes native long chromatin otherwise totally insoluble at physiological ionic strength of extracellular fluid [113, 114, 157-166]. SAP has been found also to bind apoptotic cells [154, 156, 157], the surface blebs bear chromatin fragments [114], and to nuclear debris

from necrotic cells [159, 161]. Furthermore, it has been confirmed that female SAP-deficient mice suffer higher incidence and greater severity of IC proliferative GN [98, 111].

No build-up of apoptotic bodies is detected in the glomeruli of SAP-deficient mice [159, 160]; SAP protects against breakdown of tolerance via a different mechanism most likely by stabilizing chromatin in vivo. In the absence of SAP the more aggressive degradation of insoluble chromatin may enhance its immunogenicity. Parenchymas of hepatocytes are known to be the only cells that catabolyze SAP and C-reactive protein (CRP) in vivo [115]; therefore pentraxin (CRP and SAP families) binding may be a mechanism for directing the catabolism of potential autoantigens to the liver [160-166]. The nature of autoimmunity and nephritis in SAP $^{-/-}$  mice is similar to aspects of human SLE [161-164]. Though SLE patients do have normal levels of SAP in circulation, it has been shown that the acute-phase response protein CRP, which is the human classical nonspecific acute-phase protein (APP), is decreased during 'flares' of SLE [163-165]. CRP and SAP share substantial sequence homology [162-164] and have similar tertiary fold and oligomeric assembly; together they make up the pentraxin family. In the mouse SAP rather than CRP is the main APP [164] as found in the NZB/W strain that develops spontaneous antinuclear autoimmunity and GN; NZB/W also fails to mount an acute phase response to disease activity [136, 164]. These defects probably contribute to complexities of lupus susceptibility [164, 165]. In humans CRP binds specifically to small nuclear ribonucleoprotein particles [112, 165, 166] and SAP is the only main DNA and chromatin-binding protein in the plasma in physiological states [112, 163, 165, 166]. Epitopes associated with these particles most likely contribute to the immunogenic push in SLE. This is because autoantibodies formed against double-stranded DNA (dsDNA) correlate well with tissue damage in SLE. The presence of anti-dsDNA is known to be specific for identifying SLE patients. These autoantibodies cross-react with small nuclear ribonucleoprotein particle proteins (snRNPs) [166, 167]. The later (snRNPs) are exposed on the surface blebs of apoptotic cells [158, 159]. SAP, therefore is an important component of serum with physiological role. By binding to chromatin it possibly regulates degradation of snRNPs. As such SAP plays significant part in inhibiting formation of pathogenic autoantibodies against chromatin and DNA.

In the same light deficiency of C1q complement component in humans strongly predisposes to SLE. C1q knockout mice are known to accumulate multiple apoptotic bodies in the glomeruli, C1q binds to and helps clear apoptotic cells. Most recent development in complement protein functions reveals a multitude of ligand-binding capacity for C1q (also known as p33). It is predominantly detected in mitochondria, on the

surface of microvascular endothelial cells and splenic cells [158, 167, 168]. Studies with confocal laser scanning imaging demonstrate the versatility of the binding capacity of p33. It is found on Raji cells, probably indicating a secretory pathway to get to the cell surface by this means. Outside the mitochondria on the cell surface and cytoplasm and within the nucleus C1q has been found to bind calreticulin, hyaluronic acid, thrombin, high molecular weight kininogen, fibrinogen, vitronectin, as well as hepatitis C virus core protein, protein A of *Staphylococcus aureus* and internalin B of *Listeria monocytogenes*, a ligand used by this bacteria to enter epithelial and hepatocytes. In the cytoplasm p33 binds the cytoplasmic tail of membrane type-1 matrix metalloproteinase, PKC- $\mu$ , and  $\alpha_{1B}$  adrenergic receptor [168, 169].

In the nucleus it is found to be a part of the human splicing factor-2 complex, and binds a series of viral proteins including EBNA-1 of Epstein-Barr virus, herpes simplex, and Tat and Rev of HIV. This molecule plays critical part in immune-complex recognition; its interaction with the later induces conformational changes in C1 complex resulting in the activation of the classical complement pathway. Hereditary deficiency of C1q may cause SLE and the disease state of SLE causes consumption of the protein; while progression of the SLE disease itself result in the development of anti-C1q antibodies that are particularly associated with glomerulonephritis and predominantly linked with several spontaneous murine models of SLE. The versatility of this molecule allows its universal role in many physiological as well as immunological paths that are obscure at the moment. The most revealing aspect of this molecule is its involvement in clearance of apoptotic cells. Failure to do so because of a deficiency state may lead on to autoimmune induction. For excellent details see reference [170].

The hypothesis linking complement deficiency with defective clearance of apoptotic cell is that the classical pathway deficiency contributes to the development of SLE because of an impaired capacity to clear antigen-antibody complexes in tissue damage [159,170] and release of autoantigens. This mechanism is one avenue whereby apoptotic bodies containing self epitopes can build up to overwhelm the immune system terminating in autoimmune disease. On the other hand this hypothesis endorses that C1q plays a role in the maintenance of immune tolerance through the clearance of autoantigen-containing surface blebs generated by apoptotic cells; animal models also support this. The C4 complement protein that participates in the early stages of the cascade is made of two isoforms; C4A and C4B that show considerable polymorphism and the number of C4 genes present on a haplotype does vary. The 8.1AH carries a single segment characterized by a short C4B gene and no C4A gene. This C4 defect found in 8.1AH subsets is associated with increased spontaneous release of TNF- $\alpha$  which modifies immunological parameters that predisposes to impressive number of diseases and

alterations in immune response noted in these subjects. It is however observed that in the majority of these patients, an autoimmune response clinically relevant develop only in the presence of other abnormalities; for instance in lupus severe apoptotic defect can supply a large amount of autoantigens that drive the autoimmune response [106].

#### *Generalized Lymphoproliferative Disease (gld) and Autoimmune Diseases*

*Gld* is an autosomal recessive mutational gene in the C3H/HeJ strain of mice found to be responsible for the early phase development of massive lymphoid hyperplasia with autoimmune characteristics. Immunofluorescence studies show that cells that undergo hyperplasia come from B, T, and null (Thy1<sup>-</sup>, sIg<sup>-</sup>) lymphocytes. In the presence of *gld/gld* the lymph nodes of C3H/HeJ as compared to coisogenic mice are 50-fold heavier with synthesis of antinuclear antibodies including anti dsDNA. Although the mice developed interstitial pneumonitis and developed ICs in the glomerulus, virtually all except 14% of them did not present any significant lupus-like nephritis or any vascular disease [97]. Known *lpr* characteristics include lymphoproliferation-induction in the murine species. These findings therefore confirm that in the induction of autoimmunity various phases of susceptibilities exist. The net health effect is a result of combinations of genetic factors. The mutations *gld* and *lpr* are not allelic [93, 97, 98].

#### *CD45/E2A: The Autoimmune Linkages*

Current findings point to the significant role played by the CD45 protein in the regulation of lymphoproliferation. CD45 protein, a one-pass transmembrane glycoprotein is an example of a regulated protein tyrosine phosphatase (RTP) bound to the surface of all nucleated hematopoietic cells. Each cell type produces a distinct CD45 isoform, ranging in molecular weight from 180K to 235K. The various isoforms all share the same intracellular RTPase domain, but differ in their extracellular domain concerning length and pattern of glycosylation. CD45 is important for signal transduction through antigen receptors [5-13, 172-184] that are essentially involved in the activation processes of both T and B cells by foreign antigens. The RTPs are made of a large family of widely expressed signal transduction molecules [5-7]. The primary biochemical substrates and physiological roles of most RTPs are not well characterized but the isoform characteristic for the B-cell lineage is CD45R, also known as B220 because of its molecular weight of 220K. It is suggested that CD45 act on a broad spectrum of different substrates, thereby modulating multiple receptor proximal elements positively or negatively. However extracellular antibodies also form cross-linkages with T and B cells for the induction of

polyclonal activation in these cells [5-7]. When T- and BCRs are cross-linked with extracellular antibodies the catalytic domain of CD45 is activated to delete phosphate groups from tyrosine residues found on specific proteins. Proteins such as lck, a tyrosine kinase, are then stimulated to phosphorylate other proteins in lymphocytes. It must be noted however, that most of the genes responsible for the coding of proteins necessary for intracellular signaling cascades that are activated by receptor tyrosine kinases are considered as being oncogenes in cancer cells or tumor viruses [7, 55].

A proposed model for the regulation of CD45, and by sequence homology for other receptor-like transmembrane PTPs, indicates that dimerization of these molecules suppress activities of phosphatases. Inhibition of phosphatases occurs through symmetrical interactions between an inhibitory structural wedge and the catalytic site [176]. Evidence for such proposal comes from CD45-deficient T and B cell lines that fail to respond to antigen receptor stimulation [12-14, 177, 178]. Also CD45-deficient mice show high levels of blocks in both T and B cell development and function [178]; and humans with CD45 deficiency have severe combined immunodeficiency (SCID) phenotype [179] identical to that observed in CD45-deficient mice [178]. By dephosphorylation of the C-terminal site of negative RTP within src-family kinases [8, 179, 180] CD45 retains T and B cells in a "primed" state capable of full activation on subsequent contact with antigen receptor. CD45, like all other RPTPs has an extracellular domain, a single transmembrane domain, and a cytoplasmic domain that has tandemly duplicated PTPs. Alternative splicing of exons 4, 5, and 6 gives rise to many isoforms of CD45 within the extracellular domain [8, 172-175]. The high molecular weight isoforms (CD45RA<sup>+</sup>) differs in extracellular structure and overall charge from the low molecular weight isoform (CD45RO) that is deprived of the three exons coding for O-linked glycosylation [8, 9].

T naïve cells express mainly CD45RA<sup>+</sup> isoforms that can switch to express CD45RO upon activation [8-11] (Table 2), thus indicating that the extracellular domain regulates CD45 function probably by a ligand binding or by mediating dimerization known to exist in the RPTPs such as CD45 [10] and RPTP $\alpha$ . The mechanism through which dimerization of these molecules is regulated is still evasive; chemicals like mercury with its thiol groups are capable of inducing dimerization of CD45 as well [10-14, 54, 182]. Functional testing assay of RPTP dimerization using chimeric molecule of extracellular and transmembrane domains of the epithelial growth factor (EGF) receptor fused to the cytoplasmic domain of CD45 [11] (EGFR-CD45) showed that it was capable of restoring TCR-mediated signal transduction [14, 178-180]. Rather surprisingly EGF-induced dimerization of EGFR with CD45 caused inhibition of TCR-mediated signal transduction. This inhibition occurred through dimerization of the CD45 cytoplasmic domain, indicating that the process negatively regulates CD45. Crystal structure studies demonstrated blockage of one

catalytic site by contacts with a structural wedge from the membrane-proximal region of the other [12, 13, 183, 184].

Dimerizations thus inhibit phosphatases activity, and consequently affect function through symmetrical interactions between the catalytic site and the structural wedge that contain acidic residues [14]. From these studies accurate prediction made and found to be true showed that mutations within the inhibitory structural wedge in vivo lead to inappropriate RPTP activation under normal dimerization conditions. For CD45 such dysregulated activity causes inappropriate src-kinase activation having potential pathological results. A phenotype of "knockin" mice (CD45 E613R) with a single point mutation, glutamate 613 to arginine that inactivates the inhibitory wedge of CD45 was found to cause polyclonal lymphocyte activation culminating in lymphoproliferation with severe autoimmune nephritis arising from autoantibody synthesis; and with ultimate death. Both the homozygotes and heterozygotes developed pathology consistent with genetic dominance of CD45 E613R. This model, CD45 E613R is the first reported single genetic lesion that functions in a Mendelian dominant fashion to affect SLE-like syndrome. Since most of the SLE genes are notably polymorphic alleles it is not surprising that genome analyses also identify polymorphic alleles adjacent to the CD45 locus that is weakly associated with SLE [11, 105, 122, 182].

Recently it has been shown also that lack of E2A transcription factor downstream of the TCR in the course of T-cell development in the mouse leads to increase in humoral immunity to a T-dependent antigen. E2A belongs to the basic-helix-loop-helix (bHLH) transcription factor family known to regulate cell differentiation and proliferation in many types of cells including lymphocytes [185, 186]. The bHLH domain of E2A mediates protein dimerization and DNA binding to canonical E-box DNA sequences (CANNTG) located in the enhancers of tissue-specific genes [187]. Various studies point to this molecule playing a critical role in B-cell development. Its absence in B cell halts developmental stages with complete arrest in immunoglobulin gene rearrangement though the target for its action is yet to be elucidated. This molecule has also been linked with T-cell development playing significant roles particularly in thymocyte differentiation events. Observations in E2A knockout mice indicate that there is a build-up of most immature double negative thymocytes in such animals. Genetic crosses between E2A knockout mice and RAG2 knockout mice demonstrate a role for E2A in pre-TCR selection, a checkpoint ensuring correct rearrangement and expression of the TCR $\beta$  gene during early T-cell development; it is thought that it participates in the apoptotic pathway responsible for the elimination of T cells lacking a functional pre-TCR [189, 190]. E2A is also implicated in regulating the TCR signal during positive and negative selection of double positive (DP) cells; its T cell

selectivity role was located downstream of the TCR signal and within the MAPK pathway [188, 190]. The involvement of E2A in antibody synthesis demonstrates that E2A is implicated in regulating TCR-induced T-cell proliferation events. But in naïve T and effector T cells this role seems to be reversed. In the absence of E2A, TCR-induced proliferation is increased in naïve T cells but decreased in effector T cells. At old age these mice develop antinuclear antibodies and proteinuria [191]. This indicated that E2A regulates T-cell function and the depletion of E2A may enhance the chances of age-related autoimmune diseases. The most noticeable result from these data is that the downstream targets of E2A in naïve and antigen-exposed T cells may be different or respond differently to T cell signals due to conformational changes in molecules involved.

#### *T Cell-Specific Adapter Protein Deficiency*

Mice deficient in T cell-specific adapter protein (TSAd), a T lineage-restricted signaling adaptor molecule are susceptible to lupus-like autoimmune diseases [15]. Studies with TSAd-deficient mice revealed that TSAd plays a role in the induction of T cell proliferation and T cell-mediated IL-2 secretion. Normal C57BL/6 mice are known to be nonautoimmune-prone [192]. TSAd deficiency detected in mice of C57BL/6 genetic background has been found to develop hypergammaglobulinemia with high levels of all immunoglobulin (Ig) G subclasses [15]. Large numbers of activated T and B cells in spleen that produced autoantibodies against a variety of self-targets including single stranded and double stranded DNA as well as development of glomerulonephritis were observed [192, 193]. Enhanced productions of these autoantibodies in TSAd-deficient mice were associated with defective *in vivo* cell death. This finding points to a role for apoptosis in contributing greatly to the initiation and propagation of autoimmunity. Literature data support that the overall balance between the positive and negative signaling costimulatory pathways dictates the fate of individual T cells and the immune response. PD-1 and its ligands, PD-L1 and PD-L2, like TSAd have also been demonstrated to play critical roles in regulating T cell activation and peripheral tolerance. Deficiencies of TSAd and PD-1 and ligands promote systemic autoimmunity [16-20, 53, 194].

#### *TCR/CD3-Mediated Protein-tyrosyl Phosphorylation: SLE-variant Pathways.*

The CD3, a nonpolymorphic T lineage-specific heterooligomer is noncovalently associated with the Ag-binding TCR chains, as well as with the TCR $\zeta$  chain dimer, Figures 1 and 2. The elongated cytoplasmic domains of the CD3- $\gamma$ , - $\delta$ , - $\epsilon$ , and TCR  $\zeta$  polypeptides retain the signal transducing module immunoreceptor tyrosine-based activation motif (ITAM) in one (for each CD3 chain) or three (for each TCR $\zeta$ ) copies, to a total of

10 ITAMs for each TCR/CD3 complex. The tyrosine residue within an ITAM is capable of undergoing reversible phosphorylation and forms the basis of the early signal transduction in T lymphocytes. Antigen-induced physiologic cascade of events, imitated by ligation of the TCR/CD3 complex with anti-CD3 antibody shows that the immediate phosphorylation and activation of several cytoplasmic protein tyrosine kinases (PTK) and of their substrates leads to intracytoplasmic mobilization of Ca<sup>2+</sup>, activation of key enzymes, gene transcription, cytokine synthesis, and release, proliferation, activation, or apoptosis [175-175, 193-196].

T cells derived from SLE patients have been shown to display several, frequently opposing abnormalities, involving decreased cytotoxic cell function, increased helper activity, abnormal cytokine release, and isolation of unusual T cell subpopulations [194, 195]. The TCR/CD3 signal transduction of SLE patients is known to be hyperactive in a disease-specific mode [195]. Cross-linking of the TCR/CD3 complex on fresh cells and in T cell lines led to a disease specific increase in the intracytoplasmic concentration of free Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) responses that originated from the intracellular Ca<sup>2+</sup> storage compartments. It has been also demonstrated that B cells from lupus patients undergo Ag-receptor-induced (surface IgM and IgD) signaling that also end up in disease specific increase in [Ca<sup>2+</sup>]<sub>i</sub> responses that correlated with high levels of tyrosyl phosphorylation of cellular proteins with apparent molecular size between 36 and 64 kD [22, 195-199]. Activation of the src and the ZAP-70/Syk family of PTKs is known to precede the Ag receptor-induced Ca<sup>2+</sup> fluxes and it also results in reversible tyrosyl phosphorylation of multiple protein substrates [198] yet to be identified.

Normal T cells exhibit gradual increases of protein tyrosine phosphorylation over a period of 2 minutes in almost linear mode. Immunoblotting assay after anti-CD3 mAb exposure to lupus T cells and normal patient T cells revealed that lupus T cells respond to identical treatment in a 'burst-like' fashion, achieving supranormal amounts of tyrosyl phosphorylated cellular proteins quite early within the first minute and return to levels close to baseline by the second minute. This was unlike normal controls which when exposed to the same stimulus took about 2 minutes to return to resting state [140, 141]. Isolation of receptor proteins revealed that lupus patients tend to have T cells with deficient or absent TCR $\zeta$  chain [197]. The TCR $\zeta$  was found to be absent on T cell subsets from lupus patients including CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD45RO<sup>+</sup>, and CD16<sup>+</sup> cells by flow cytometry measurements. Detailed scrutiny revealed that where T cell deficiency existed CD4<sup>+</sup> were not affected but CD8<sup>+</sup> and CD16<sup>+</sup> cell subsets were deficient of  $\zeta$  chains. Functionally, deficiencies or defects in the  $\zeta$  chain may contribute to the numerous immune cell abnormalities that seem to predominantly involve the CD8<sup>+</sup> and NK cells in lupus. The TCR $\zeta$  forms a homodimer that is associated with the TCR/CD3

complex. Functions performed by  $\zeta$  chain include roles in signaling in mature T cells, in appropriate assembly and transfer from the cytoplasm to the cell surface of the complete TCR/CD3 heterooligomer, and in normal development and selection of immature thymocytes [196-201]. The long cytoplasmic domain of each  $\zeta$  chain has 3 ITAM modules that participate in signaling. Src family of PTK phosphorylates these ITAMs by forming anchoring sites for the tandem SH2-domains of ZAP-70, a required PTK important for propagation of ongoing signals [200, 202]. Analysis of lupus T cells indicates that  $\zeta$  deficiency does not necessarily stop signal transduction because other molecules can substitute for the continuous signal propagation. However, the outcome may be different. The  $\zeta$ -knockout mice transfected with the Fc $\epsilon$ RI $\gamma$  gene have normal T cell development and function [198, 200, 201]. Murine knockout models do not express the Fc $\epsilon$ RI $\gamma$  or TCR $\eta$  and TCR $\zeta$ , yet the cells mature to become T cells [197-201]. This implies that the presence of TCR $\zeta$  chain may not be an absolute requirement for the induction and flow of the signal transduction process initiated by the TCR/CD3 complex in mature or immature T cells, or for the appropriate gathering and release to the surface of TCR/CD3 polypeptides (Figures 2 and 3). TCR $\zeta$ -deficient T cells can initiate and propagate the signal transduction cascade as far as they express adequate amounts of TCR/CD3. Under such conditions signaling can be transduced using other ITAMs from other invariant chains of the CD3 complex such as the ZAP-70 and Syk. Even though signal transduction still occurs with Ag receptor signaling in  $\zeta$ -less cells, the outcome is different. Tyrosyl phosphorylation observed under such conditions is altered, and the production of IL-2 is decreased [202-205]. These findings are well known to occur in lupus patients;  $\zeta$ -less NK cells in lupus patients are also defective in function. One significant effect of the absent or deficient  $\zeta$  chain on lupus T cells is the decline in positive and negative selection of the thymus-dependent cells important to weed out self-reacting T lymphocytes. In mature T cells the TCR $\zeta$  chain is singularly effective in inducing apoptosis [205-209]; and this mode of TCR/CD3-mediated apoptotic cell death is known to be decreased in peripheral T cells of SLE patients in a disease-specific way [194, 195] that is explainable by the  $\zeta$ -less T lymphocytes in these patients [140]. Thus, abnormalities in the expression of the TCR $\zeta$  protein may be a common factor in signal dysregulation and in the malfunction of  $\zeta$  chain-associated immune cells in SLE. The gene for TCR $\zeta$  has also been located on chromosome 1 in lupus patients [103] and further characterization will help elucidate the mechanisms involved in this elusive disease.

### Apoptosis and Autoimmunity

Apoptosis and related pathways that lead to cell death have significant roles to play in the development of

autoimmune diseases. The immune system presents avenues for how programmed cell death can regulate self and non-self recognizing machinery. Dysregulation of apoptosis has been associated with and may even be central in several disease pathogenesis. Too little apoptosis may end up in cancer, autoimmune or other chronic inflammatory diseases, while enhanced apoptosis is implicated in the stroke-induced neuronal damage and neurodegenerative disorders [210-212].

### The Apoptotic Machinery

Many gene products operating within the apoptotic pathways have been implicated in autoimmunopathogenesis when deficient or abnormal in function. The machinery within which apoptosis function engages a number of molecules among which are death receptors (DR). These DRs are unique sensors located on the surface membranes in certain cells. They are capable of detecting extracellular fatal signals and respond by activating the cell's intrinsic apoptosis machinery. The Bcl-2, an antiapoptotic molecule plays a pivotal role in influencing the survival or demise of T cells [213]. This molecule participates in the mitochondrial pathway leading to death and is downregulated after T-cell expansion. This indicates that highly differentiated T cells are very susceptible to apoptosis (Figure 2) [210]. However, certain groups of cytokines can prevent T cell apoptosis and do so by re-inducing Bcl-2 expression [211, 213]. Another group of cell surface molecules related to the TNF receptor do also trigger T cell apoptosis if ligated at the period of T-cell activation in the absence of costimulatory signals. This phenomenon is known as activation-induced cell death [210] or AICD. The CD95, also called Fas or Apo1 and TNFR1 (or p55 or CD120a) are the best-characterized DRs known to utilize AICDs. Others include DR3 (also called Apo3), WSL-1, TRAMP, or LARD; DR4 and DR5, (also called Apo2), TRAIL-R2, TRICK 2, or KILLER [211]. CD95 expression on T cells progressively increases as a T cell also progressively differentiates; table 2. Mammals can actively direct individual cells to self-destruct by AICD. This "instructive" apoptosis is essential particularly in the immune system [214] Specific death receptors/cell surface receptors are able to initiate apoptosis signals and play central roles in instructive apoptosis. These ligands can start off death caspases within seconds of binding and death of the cell can take a matter of hours. Death receptors are found also among the tumor necrosis factor (TNF) receptor gene superfamily that is defined by cysteine-rich extracellular domains [209, 210, 215]. In addition they also have a homologous cytoplasmic sequence called the "death domain" [210, 211, 216]. While these domains commit cells to death, other functions are assigned to the sequence. These include even the capability to counteract apoptosis itself. In humans, freshly isolated CD4<sup>+</sup>CD25<sup>+</sup> are sensitive to cytokine starvation-induced apoptosis and this is related to low expression of Bcl-2; table 2. Apoptosis of these

cells can be stopped by the addition of interleukin-2 receptor (IL-2R)  $\gamma$  chain signaling cytokines, such as IL-2 and IL-15, and also IFN- $\alpha/\beta$  [217] indicating that regulatory activity of these cells can be maintained in the presence of exogenous factors to prevent their apoptosis. This explains why defective CD95 and/or ligand CD95L may not respond to these exogenous factors and spontaneously undergo AICD.

#### *CD95 and Ligand Dysfunctions Lead to Autoimmune Disease*

Dysregulation of the Fas-mediated apoptosis is a known factor contributing to the rise of autoimmune disorders [73-75, 218, 219]. Some of these proteins initiate apoptosis. The importance of CD95 in immunoregulation is evidenced by homozygous defect in the CD95 found in *lpr* and *gld* genes in mice. Homozygous mutations of the Fas or FasL gene respectively affect Fas-induced PCD and cause lymphoproliferation and a generalized autoimmune disease state. MRL<sup>+/+</sup> mice undergo autoimmune proliferative syndrome characterized by massive lymphadenopathy and lupus-like immunopathology. Pathogenesis of the disease is more severe and detected early in MRL *lpr/lpr* mice with the homozygous CD95 defects [72, 73]. Similarly humans who have CD95 mutations with *lpr*-like genetic constitution also suffer from a variety of lymphoproliferative autoimmune diseases [73-76, 218]. These patients also have defects downstream in the CD95 death pathway. CD95 and CD95L are implicated also in pathological suppression of immune surveillance, that is, elimination of tumor-reactive immune cells by certain tumors that constitutively express CD95L. Thus, defects in the apoptotic-signaling pathway appear to be linked to susceptibility to autoimmune diseases.

CD95 and CD95L are involved in three physiologic types of apoptosis:

- 1) peripheral deletion of activated T cells at the end of an immune response,
- 2) the destruction of targets such as virus-invaded cells or cancer cells by cytotoxic T cells and by natural killer cells, and
- 3) elimination of inflammatory cells at areas such as the eye thought to be 'immune-privileged'.

Mutations in the CD95 or CD95L can lead to accumulation of peripheral lymphoid cells and to a fatal autoimmune syndrome with described characteristics that culminate in the enlargement of the lymph nodes. The same CD95 and CD95L are involved in pathological suppression of immune surveillance that lead to destruction of tumor-reactive immune cells by certain tumors that constitutively express CD95L [216, 217]. CD95L, like other TNF members is a homotrimeric molecule. Each CD95L can bind to three CD95 molecules leading to clustering of the receptor's death domains (trimerization) [220]. The death effector domain is an example of a more widely distributed homophilic

interaction called caspase recruitment domain (CARD) found in many caspases with large prodomains including caspases-2, -8, -9 and -10 [214]. Attachment of Fas-associated death domain (FADD), an adapter protein to caspase-8 leads to oligomers that self cleave caspase-8 for activation. Caspase-8 carries out the activation to downstream caspases such as caspase-9, the mammalian functional homolog of CED-3, committing the cell to apoptosis. Activated macrophages and T cells are the predominant cells that produce TNF during infections. TNF also trimerizes with TNFR1, activates the transcription factors NF- $\kappa$ B and AP-1 leading to induction of proinflammatory and immunomodulatory genes [219]. In some cell types TNF also induces apoptosis via TNFR1.

These multi-pathways utilized by Fas signaling could be the source of the various effects of Fas ligation in several cell types. For instance, Fas triggering mainly induces PCD in activated T cells, but is costimulatory in resting T cells [212, 218]. Molecules participating in Fas-signaling are also involved in signaling via other surface receptors. Sphingomyelinase is known to be involved in signaling via several cytokine receptors. Such observations prompted Dianzani and his group [219] to postulate that alterations at different levels in the Fas signaling routes may induce clinical patterns that only partially overlap, as shown in mice carrying the *lpr* or the *me<sup>v</sup>* gene mutations. Patients with autoimmune/lymphoproliferative disease (ALD) may be heterogenous with mutations at different levels of the Fas signaling pathway. Cases of children with ALD have been found to have chronic thrombocytopenia, serum autoantibodies, and lymphadenopathy and/or splenomegaly. T-cell lines from some of these ALD patients were relatively resistant to PCD induced by monoclonal antibodies to Fas. But typical patients with chronic idiopathic thrombocytopenic purpura (ITP) showed no lymphadenopathy. DNA sequencing of the Fas gene did not reveal any causal mutation in patients with ALD [71-75, 219].

Therefore this distinguished them from patients with the human autoimmune lymphoproliferative disease syndrome (ALPS) who carry mutations of the *Fas* gene. In addition ALD patients do not demonstrate peripheral expansions of CD4<sup>+</sup>/CD8<sup>-</sup> (double-negative) T cells known to be characteristic of ALPS phenotypes. Ceramide-induced PCD was found defective in ALD patients but not in patients with typical chronic ITP. These data indicate that the defect associated with ALD patients may operate via the Fas signaling pathway downstream from the sphingomyelinase and that Fas gene mutations and double negative T-cell expansion are not the only signs of defective Fas system. Expression of ALD probably depends on the type and severity of mutations and the concomitant presence of mutations located at different levels of the pathway, a scheme that is in conformity with multiple checkpoint notions.

A variety of stimuli induce apoptosis. These include depletion of growth factors, hormones,

xenobiotics like mercury, gold, silver, heat-shock proteins,  $\gamma$ -irradiation and cross-linking of Fas antigen [29, 42, 81]. Apoptotic signal transduction pathways activated by various treatments converge into a single path driven by *ICE/Ced-3* family caspases and negatively regulated by anti-cell death proteins such as the *Bcl-2/Ced-9* and the IAP families [214 - 218]. But the biochemical mechanisms of these anti-apoptotic proteins remain to be elucidated. Known mammalian *ICE*-related genes: *ICE (caspase-1) (-like)*, *Ich-1 (caspase-2) (-like)*, and *CPP32/Yama (caspase-3) (-like)* proteases have been implicated in apoptosis, initially based on inhibition of apoptosis by their synthetic or natural inhibitors. Caspases are believed to behave as zymogens, protease cascade that cleave other members of the family *in vitro* to activate them. The sequential activation of caspase 1 (-like) and caspase 3(-like) proteases is needed in *in vivo* apoptosis induced by different stimuli including Fas (*CD95*) stimulation, VP16 and calcium ionophore [214].

Cell death normally occurs after proteolysis of intracellular proteins, including nuclear auto-antigens. Apoptotic bodies contain several self-epitopes, as well as many components comprising auto-antigenic macromolecular complexes [42, 72-75] and these molecules are targets for multiple autoantibody responses. But apoptosis is believed to be a non-inflammatory process, and the processing and presentation of self-Ags as a result of apoptotic cell death most likely end up in cell elimination for tolerance induction, than stimulation, of auto-reactive cells [212, 218, 221]. Autoimmunity is therefore considered more likely to be associated with defective PCD, such as the acceleration of systemic autoimmunity found in mice with the *lpr* or *gld* mutations [72].

### Autoimmunity and Cognitive Problems

Human SLE is characterized by specific formation of antinuclear antibodies particularly against double-stranded (ds DNA). However, dsDNA is known to be a very poor immunogen and depends on T helper cells for immune responses. All indications point to nucleosomes as the principal autoantigen in SLE and that DNA circulates in SLE patients in the form of nucleosides. Research confirms that nucleosides are generated through apoptosis [221]. Increasing evidence shows that pathways associated with apoptosis is dysfunctional in human SLE patients [210, 220]. Nucleosomes have high affinity for heparin sulfate in the glomerular basement membrane (GBM). Through complex formation with nucleosomes, antinuclear antibodies (both nucleosome-specific and anti-dsDNA autoantibodies) acquire a high affinity for the GBM. This represents an important initiation episode in the course of lupus nephritis development.

It has been demonstrated that the pentapeptide Asp/Glu-Trp-Asp/Glu-Tyr-Ser/Gly is a molecular mimic of dsDNA. This sequence is also found in the extracellular domain of murine and human NMDA (N-

methyl-D-aspartate) receptor subunits NR2a and NR2b. The NR2 receptors do cross-react with both murine and human anti-DNA antibodies to mediate apoptotic death of neurons *in vivo* and *in vitro* [221, 223]. Lupus antibodies cross-react with DNA and NMDA receptors also, and they cross the blood brain barrier (BBB) and possibly mediate non-thrombotic and non-vasculitic abnormalities of the central nervous system [222, 224]. Nearly 80% of patients with lupus disease are known to undergo neuropsychiatric symptoms and cognitive deficiency [225-227].

These neurologic representations are clinically associated with reactions from autoantibodies and highly so with those formed against native DNA [224-232]. NR2 receptors that bind murine and human anti-DNA bind to glutamate, an excitatory neurotransmitter located in the forebrain [223, 228-230] and known to have a role in learning and memory [226, 234], neural development, aging, and responses to the environmental stimuli. Heightened and continuous stimulation of NR2 receptors can result in excitotoxic neuron death from excess invasion of  $Ca^{++}$  into cells [234]. A glutamate ligand antagonist, phenylcyclohexylpiperidine binds to NR2s and act as psychotomimetic that induces hallucinations and paranoia. Attachments of antibodies to NR2a or NR2b mediate some of the CNS dysfunctions noted in SLE patients [226] by causing apoptotic death of neurons.

### Conclusions

There is sufficient evidence that self-molecules that carry tissue-restricted expression are expressed in the thymus. This provides means for the development of self-tolerance. Specific genes in individual tissues or organs express specific molecules that may be associated with that tissue/organ alone. This is true because most cellular specializations involve turning off and activating particular genes necessary for the day-to-day function of the organ. The beta cells of Langerhans are insulin expressors and their function will be specified for the sole expression of this hormone. This hormone needs to be in circulation to function. Data indicate that the thymus and peripheral lymphoid tissues also express these molecules. Thus the thymus is capable of eliminating cells that carry self-expressing epitopes in high quantity by negative selection. If this notion is expanded to all tissues and organs one can envisage how the thymus and individual organs play significant two-way role in maintenance of tolerance. The mechanisms of positive and negative selection in the thymus play major roles in shaping self-tolerant T-cell repertoire particularly in early life during the maturation of the immune system.

Developing lymphocytes that do not express high reactivity against self-peptides are positively selected in the cortex of the thymus and enter the circulation as mature lymphocytes. Deletion of lymphocytes carrying

high levels of self-peptides occurs in the thymic medulla. This centrally expressed tolerance to self-molecules is carried out a step further for the continued maintenance of tolerance. Peripheral tolerance mechanisms operate in extrathymic lymphoid tissues that also involve deletion, anergy, regulatory cells that contribute to the maintenance of autoreactive cells under rigid control. Presentations of self-molecules that carry tissue-specific pattern of expression would be mainly a peripheral event and depend on the capture of such self-molecules by APCs, in particular immature dendritic cells (DC). However it is becoming clear that most molecules, including many with tissue-restricted expression (or 'peripheral' proteins), are also located in the thymus. The expression of these proteins correlates, mostly with the presence of the corresponding transcript in the thymus. Models derived from transgenic and knockout mice, which express altered insulin genes are examples of tolerogenic role of self-antigen expression in the thymus and peripheral lymphoid tissues.

These models also assist in deriving mechanisms underlying autoimmune responses. The initiation of autoimmune disease in any organ or tissue will provide in the immediate time period epitopes characteristic of the tissue/organ dependent upon genes expressed in the locale. Therefore in any disease commencement one would believe the tissue/organ specific proteins will be the starting targets for tolerance breakage. Once tolerance is broken mechanisms must exist to induce total body breakdown of tolerance. It is suggested that products of the nucleus provide such means for total body break in tolerance. This is evidenced by the fact that most autoimmune states arrive at developing ANA in addition to specific tissue/organ determining autoantibodies. The etiology of autoimmune diseases is multifactorial and involves polygenes or scattered genes that seem to operate in a Mendelian fashion with environmental influences. Alterations occurring in various genetic pathways seem to have contributions to the degree of susceptibility to the autoimmune state. While only single defects are discussed one can easily envision a situation whereby multiple defects can exist in any specific disease.

Genetic and environmental exposures to xenobiotics in particular may influence different functions of immune cells. Molecules that regulate the immune system and the homeostatic environment in the course of maturation of the latter cause disorganization of biochemical, and physiological states of autoimmune-prone individual. This is demonstrated with CD4<sup>+</sup>CD25<sup>+</sup> Treg cells that acquire higher levels of CD95 receptors with maturation while receptors for the protective Bcr gene subside. Many of the defects discussed may singly or combine to contribute in various ways to a disease state. It appears that no single gene is sufficient for the onset of disease and in any individual, disease vulnerability and presentation in terms of specific organ severity and prognosis is a result of additive or epistatic effects of numerous accidentally inherited alleles, many of which

may be common to other autoimmune diseases. Up to date there are over 247 different types of the cluster of determinant antigens used by immune cells for regulatory purposes. As more of these antigens are evaluated and functions revealed, the mechanisms of autoimmune initiation and pathogenesis will become clarified. Yet one can hypothesize from published studies that all pathways most likely lead to apoptotic and/or necrotic demise of cellular components particularly the nuclear materials that seem to accompany most immune dysfunctions. Nuclear targets seem to be a link to a general and total body loss of tolerance to self-proteins.

Studies in genetic penetrance reaffirm that superimposed upon genetically complex susceptibility are aging processes that affect various immune organs such as the thymus or the lymphoid stem cells and their internal control of self-reactivity. The prevalence of some autoantibodies and MHC class across-board for most of the autoimmune diseases is a clear indication that most autoimmune diseases have a common mechanism at arriving at a disease state although the initial pathway chosen may be very dependent on the immunogen or epitope doing the stimulation. For instance DR3 haplotype components are distributed among patients of SLE, scleroderma (CREST), celiac disease, Hashimoto's thyroiditis, Graves disease, multiple sclerosis, myasthenia gravis, IDDM, and individuals suffering from polymyositis-dermatomyositis. Such observations must infer that individuals with such a particular haplotype develop disease dependent on the overall contribution from regulatory molecules, causal agent(s), dose and the environmental conditions. The inducing agent must dictate the type of disease resulting from any exposure since it is only through its epitope that the immune system will respond. Most autoimmune disorders present as chronic symptoms and are important causes of morbidity among diseases prevalent in American society; they do have indeed a greater impact on public health as to cost to the U.S. health system in terms of diagnosis and health care delivery. This calls for novel therapeutic approaches as more information becomes available through research and clinical studies. Available information on RA patient management is a helpful start. However caution is necessary for the direct usage of cytokines that may display multiple effects. Besides cytokines have effects on major cells in the autoimmune system. Patients with active disease have cells that are responding abnormally to stimulants because of either deficiency in cell receptors and/or plasma proteins. Injections of extraneously derived cytokines need to be targeted to an environment with normal tissue/organ function. Perhaps genetic engineering may overcome these problems. Introducing a gene that expresses the normal product is a means of regenerating normalcy.

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