

Alterations in the expression of signal-transducing CD3 ζ chain in T cells from patients with chronic inflammatory/autoimmune diseases

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Abstract

The CD3 ζ chain, a component of the T cell receptor (TCR)/CD3 complex, is considered to be a limiting factor in the assembly and transport of the TCR/CD3 complex to the cell surface and is crucial to receptor signaling function. Recent studies have demonstrated altered expression and function of this signal transduction molecule in T and natural killer cells in patients with chronic inflammatory/autoimmune diseases. In this review, current knowledge concerning the expression of CD3 ζ chain as well as the mechanisms responsible for abnormal expression of this molecule in systemic lupus erythematosus, rheumatoid arthritis, and childhood idiopathic nephrotic syndrome are summarized.

Key words: CD3 ζ chain, TCR/CD3 complex, chronic inflammatory diseases, autoimmune diseases.

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INTRODUCTION

Antigen-specific T cells play a central role in immune and inflammatory responses. An appropriate immune response of these cells depends on the careful regulation of their activation. It has been very well documented that for optimal activation and induction of their differentiation into effector and memory states, T cells require two independent signals: one mediated by the T cell receptor (TCR)/CD3 complex and the other transduced via costimulatory receptors [96, reviewed in 81].

OVERVIEW OF TCR/CD3 STRUCTURE AND THE SIGNAL TRANSDUCTION CASCADE

The TCR/CD3 complex is one of the most intricate membrane receptor structures because it consists of six distinct chains [reviewed in 8, 50]. The clonotypic α and β chains of the TCR (the $\alpha\beta$ TCR heterodimer) are responsible for recognizing antigen embedded in the

major histocompatibility complex (MHC) molecule present on the surface of antigen-presenting cells (APCs). The remaining, invariant subunits, collectively called the CD3 complex, include the CD3 γ , δ , ϵ , and ζ chains (also referred to as the TCR ζ chain). These subunits are known to form three distinct dimers: CD3 $\gamma\epsilon$, CD3 $\delta\epsilon$, and CD3 ζ - ζ . A single $\alpha\beta$ TCR heterodimer is noncovalently associated with one copy of each of the CD3 $\gamma\epsilon$, CD3 $\delta\epsilon$, and CD3 ζ - ζ dimers [reviewed in 8]. Ligation of the $\alpha\beta$ TCR chain with its cognate peptide/MHC ligand expressed on APCs results in the rapid phosphorylation of the two tyrosine residues within immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 γ , δ , ϵ , and ζ chains by members of Src family kinases, Lck and Fyn [96, reviewed in 9, 38, 76, 81, 107]. Fully phosphorylated, CD3 ζ , γ , δ , and ϵ ITAMs function as docking sites for protein tyrosine kinases of the Syk family, such as ZAP-70. The phosphorylation of critical adapter proteins, such as the transmembrane adapter protein linker for the activation of T cells (LAT), the Src homology 2(SH2)-domain-containing leukocyte protein (SLP)-76, and phospholipase (PLC) γ 1, by ZAP-70 and

Src kinases then serves as a link between membrane-proximal phosphorylation events and the activation of downstream signaling pathways [reviewed in 52]. Two major downstream signaling pathways are activated as a result of the events described above, namely the Ras/Raf/mitogen-activated protein kinase and the PLC γ /calcium/calcineurin pathways [96, reviewed in 9, 38, 76, 81, 107]. Ultimately, these two pathways converge in the nucleus, where transcription factors such as AP-1 and NFAT initiate cytokine gene transcription, resulting in T cell proliferation and effector responses (see Fig. 1).

Based on the structural model of the TCR/CD3 complex, the CD3 ζ chain is presumed to be the dominant signaling component of this complex, as it carries three ITAMs, whereas the CD3 γ , δ , and ϵ molecules contain a single ITAM [reviewed in 55, 74]. This notion

is supported by the fact that CD3 ζ chain is one of the earliest and most heavily tyrosine-phosphorylated molecules evident immediately after receptor ligation [reviewed in 75]. CD3 ζ chain can appear as two predominant tyrosine-phosphorylated forms of 21 and 23 kDa [reviewed in 55, 74, 75]. It has been determined that the 21-kDa form of CD3 ζ is generated by phosphorylation of four tyrosine residues located in the two membrane-distal ITAMs and is bound by an inactive pool of ZAP-70 (see Fig. 2). Phosphorylation of all six tyrosines in the three CD3 ζ chain ITAMs results in generation of the 23-kDa form of CD3 ζ chain, which favors ZAP-70 activation and consequently leads to full T cell activation.

Interestingly, the 21-kDa form of CD3 ζ chain is the only ITAM-containing subunit of the TCR/CD3 com-

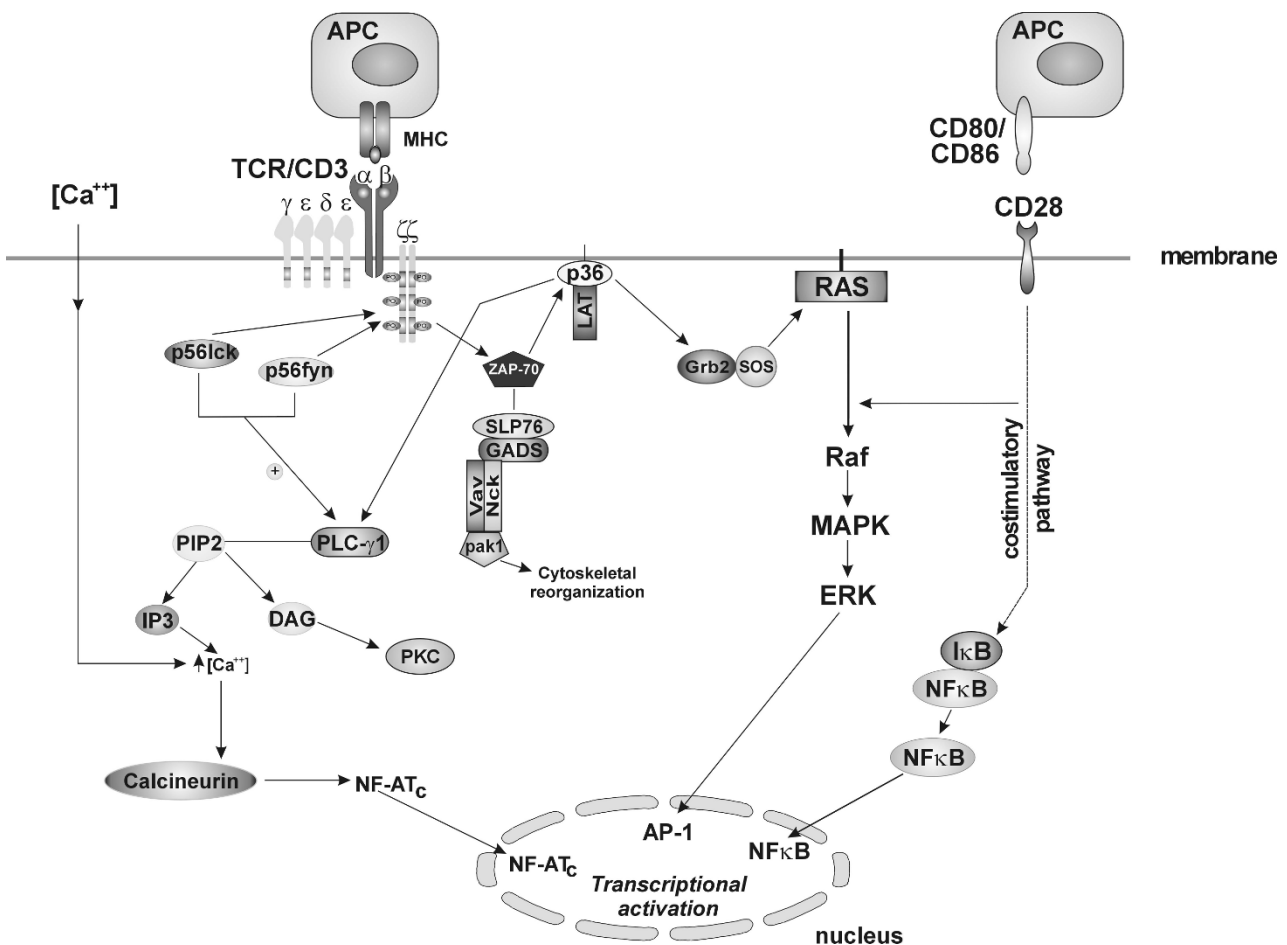


Fig. 1. TCR signal transduction pathways. Following TCR engagement, members of Src family kinases, Lck and Fyn, are activated, leading to the phosphorylation of tandemly arranged tyrosine residues within the ITAMs of the CD3 γ , δ , ϵ , and ζ chains. Phosphorylated ITAMs in the CD3 γ , δ , ϵ , and ζ chains function as docking sites for the recruitment of ZAP-70, which subsequently phosphorylates an adapter protein, such as LAT and SLP-76. The phosphorylation of adapter proteins initiates the Ras/Raf/MAPK (ERK) and PLC γ /calcineurin/calcium pathways and promotes the formation of the SLP-76-Vav-Nck-Pak1 complex, which may be important in the regulation of cytoskeletal rearrangements. Sustained activation of these pathways is required for transactivation of the transcription factor AP-1 complex and NF-AT, leading to IL-2 production, T cell proliferation, and effector responses. LAT: linker for the activation of T cells; SLP76: Src homology 2 (SH₂)-domain-containing leukocyte protein-76; PLC- γ 1: phospholipase C γ 1; PIP2: phosphatidylinositol 4,5 bisphosphate; IP3: inositol 3,4,5 trisphosphate; PKC: protein kinase C; DAG: diacylglycerol; Grb2: growth-factor-receptor-bound protein 2; SOS: Ras GTPase guanine nucleotide exchange factor; GADS: Grb2-related adapter protein; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinases; NFATc: nuclear factor of activated T cells; AP-1: activating protein 1; NF κ B: nuclear factor- κ B; I κ B: inhibitor of nuclear factor- κ B.

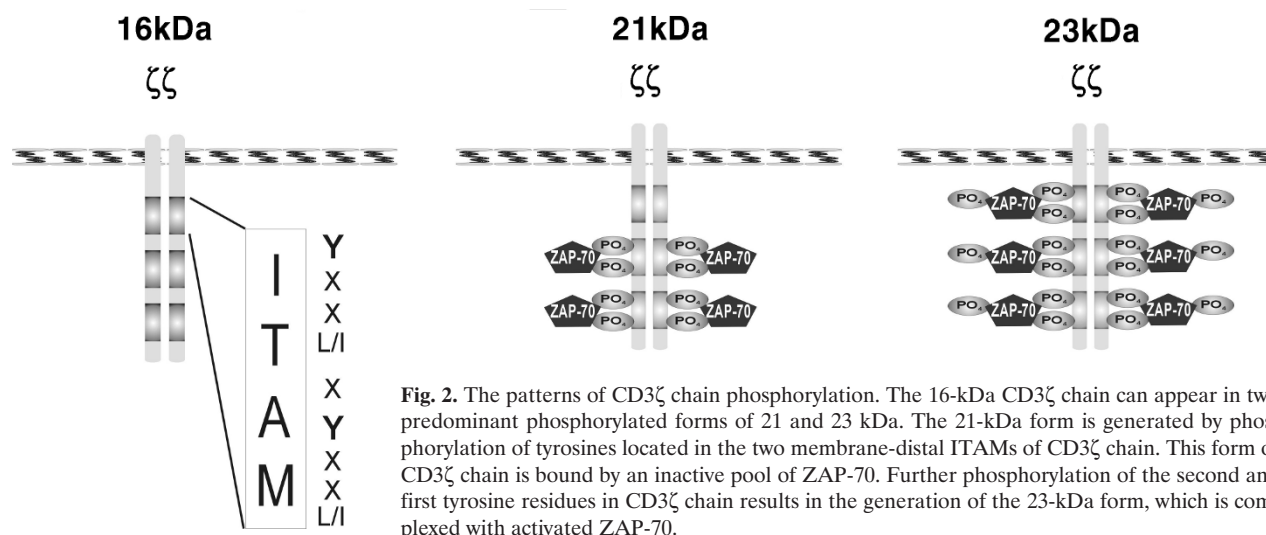


Fig. 2. The patterns of CD3 ζ chain phosphorylation. The 16-kDa CD3 ζ chain can appear in two predominant phosphorylated forms of 21 and 23 kDa. The 21-kDa form is generated by phosphorylation of tyrosines located in the two membrane-distal ITAMs of CD3 ζ chain. This form of CD3 ζ chain is bound by an inactive pool of ZAP-70. Further phosphorylation of the second and first tyrosine residues in CD3 ζ chain results in the generation of the 23-kDa form, which is complexed with activated ZAP-70.

plex existing in a constitutively phosphorylated state when isolated from thymocytes and peripheral T cells [reviewed in 74, 75]. The functional role of the 21-kDa form of CD3 ζ chain is not fully clarified. A role for this form of CD3 ζ chain in the processes of autoimmunity has recently been suggested [73, 82]. Pitcher et al. [73] provided direct evidence that the selective expression of the 21-kDa form, in the absence of the 23-kDa form of CD3 ζ chain, modifies negative selection in HY/YF1,2 male mice, facilitating the development of potentially autoreactive T cells. Further studies are required to understand how the 21-kDa form of CD3 ζ chain contributes to autoimmunity at the molecular level.

MOLECULAR CHANGES IN THE TCR/CD3 COMPLEX DURING DIFFERENTIATION OF HUMAN NAÏVE AND MEMORY CD4⁺ T CELLS INTO HUMAN EFFECTOR CD4⁺ T CELLS

The initiation of adaptive immune responses depends on the activation and differentiation of naïve T cells into effector T cells. Effector T cells migrate to antigenic sites, where they orchestrate an inflammatory response that can either protect against infection or, sometimes, contribute to pathologies in autoimmune diseases (such as systemic lupus erythematosus (SLE) [64], rheumatoid arthritis (RA) [71], transplant rejection [78], and chronic viral infections [98]). Thus an explanation of the biochemical processes taking place in the effector T cell subset seems to be essential to understand its role in normal and pathological immune states. Recent studies revealed interesting features of TCR/CD3-mediated signaling in effector CD4⁺ T lymphocytes that have implications for effector T cell function [48, 49]. Krishnan et al. [48] found that effector CD4⁺ T cells, generated by anti-CD3 or antigenic stimuli, exhibited increases in tyrosine phosphorylation of

TCR signaling intermediates and interferon γ production, which suggests an augmentation of intracellular signals consistent with potent effector cell functions. Paradoxically, despite this biochemical hyperactivity, expression of CD3 ζ and CD3 ϵ protein was greatly reduced coincident with a reduction in surface TCR/CD3 expression [48]. The profound loss of CD3 ζ chain in effector CD4⁺ T cells, the critical signaling intermediate responsible for inducing the downstream phosphorylation cascades, concomitant with an increase in total intracellular tyrosine phosphorylation suggested that TCR/CD3-coupled signals may be transduced through an alternative signaling subunit in this subset of lymphocytes. Biochemical studies seem to confirm this hypothesis. Krishnan et al. [49] found a dramatic up-regulation of FcR γ protein in effector CD4⁺ T cells. FcR γ protein, originally identified as a subunit of the high-affinity IgE receptor, is structurally and functionally homologous to CD3 ζ chain [reviewed in 77]. FcR γ can replace the signaling function of CD3 ζ chain because it contains an ITAM. Unlike CD3 ζ chain, which mediates signaling by associating through ZAP-70, FcR γ mediates signaling by associating with the phosphorylated protein kinase Syk [85, 86]. Krishnan et al. [49] found that effector CD4⁺ T cells exhibit a striking up-regulation of Syk kinase and its phosphorylation, whereas ZAP-70 is admittedly expressed, but is not phosphorylated. Based on these findings, Krishnan et al. [49] proposed that human effector CD4⁺ T cell differentiation is accompanied by a physiological switch in the TCR/CD3 signaling complex, which consists of a change of the classical TCR/CD3 ϵ /CD3 ζ /ZAP-70 complex in primary resting CD4⁺ T cells to an alternate TCR/CD3 ϵ /FcR γ /Syk complex in effector CD4⁺ T cells (see Fig. 3). It is interesting that this physiological switch in the TCR/CD3 signaling complex is not observed in CD8⁺ T cells [42]. Kersh et al. [42] found that effector and naïve CD8⁺ T cells express an equivalent amount of CD3 ζ chain, and in effector CD8⁺ T cells ZAP-70 is

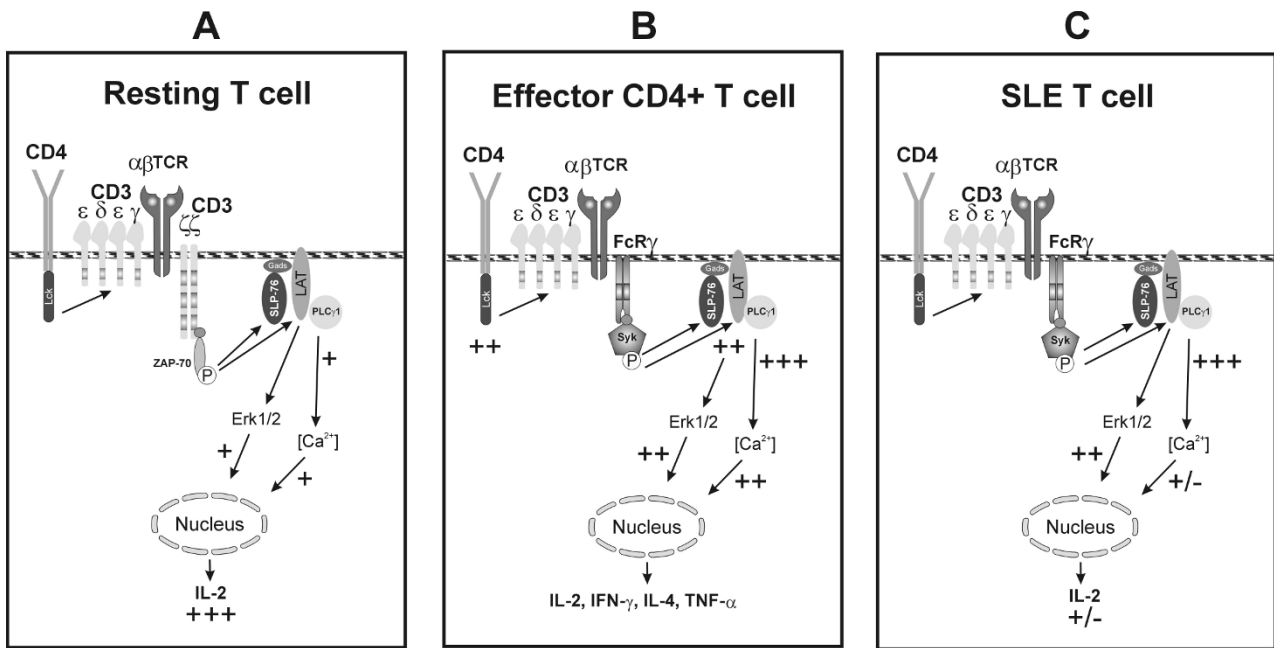


Fig. 3. TCR signal-transduction pathways in resting T cells, effector CD4⁺ T cells, and T cells from patients with systemic lupus erythematosus (according to Krishnan et al. [46], copyright 2003 The American Association of Immunologists, Inc). The classical TCR/CD3/CD3 ζ /ZAP-70 complex in resting CD4⁺ T cells from normal individuals (panel A) is replaced by TCR/CD3/FcR γ /Syk during CD4⁺ T cell differentiation to become effector CD4⁺ T cells (panel B). The similarities between effector CD4⁺ T cells of normal individuals (panel B) and freshly isolated peripheral blood T cells from patients with SLE (panel C) suggest that the altered signaling pattern observed in T cells from patients with SLE may partially represent the effector CD4⁺ T cell phenotype. P: phosphorylation status; +: increase in activity; -: decrease in activity.

activated. Therefore, biochemical studies revealed the occurrence of marked differences in the TCR/CD3 signaling pathway of human effector CD4⁺ and CD8⁺ T cells.

It is noteworthy that the down-regulation of CD3 ζ chain expression is reversible when effector CD4⁺ T cells are removed from the activating stimulus [48]. This finding suggests that loss of CD3 ζ chain expression may be a feature of chronic T cell activation and effector CD4⁺ T cell generation *in vivo* [48, 49]. It is therefore an intriguing question whether the alterations in CD3 ζ chain expression are observed in patients with diseases marked by chronic immune activation.

SYSTEMIC LUPUS ERYTHEMATOSUS

T cell abnormalities in patients with SLE

SLE is a multisystem disease characterized by the abundant production of a variety of autoantibodies to nuclear antigens and cell-surface and serum proteins [reviewed in 15, 45, 60]. Uncontrolled production of autoantibodies by B cells leads to the formation of immune complexes which can be deposited in various tissues, resulting in organ dysfunction and the clinical manifestation of SLE. Many immune-cell populations take part in this process. T cells are considered to be central to the pathogenesis of SLE because a dysfunction

in their regulatory function may account for the altered immune response and overproduction of pathogenic autoantibodies [reviewed in 45]. In fact, increased numbers of circulating activated HLA-DR⁺CD4⁺ T cells were found in patients with SLE and the proportion of these cells positively correlated with serum levels of IgG in these patients [111]. Moreover, many abnormalities of peripheral blood T cells from SLE patients, including T lymphocytopenia, low proliferative responses to lectin, anti-CD3 and anti-CD2 stimulation, and lower production of Th1-type cytokines such as interleukin (IL)-2, have been reported [reviewed in 93, 94]. Additionally, in contrast to the up-regulated expressions of adhesion molecules and their downstream signaling molecules, the pathway involving the TCR/CD3 signaling complex appears to be down-regulated [reviewed in 37, 93, 94]. The altered response of peripheral blood T cells from patients with SLE reverts to normal, or even much higher than normal, when the cells are stimulated with phorbol ester and ionomycin, which directly activate a protein kinase C located downstream from the TCR/CD3 complex. This finding could indicate that a potential defect may reside in the proximal signal transduction around the TCR/CD3 complex [reviewed in 93, 94]. In fact, biochemical and molecular studies showed that 70% of patients with SLE exhibit a decrease in tyrosine-phosphorylation of CD3 ζ chain, 55% have decreased expression of this protein compared with healthy subjects and patients with other systemic

rheumatic diseases, and 30% carry some CD3 ζ chain mRNA aberrations [6, 53, 72, 95, reviewed in 94]. Despite the decreased expression of the CD3 ζ chain, cross-linking of the TCR/CD3 complex leads to a disease-specific increase in the free intracytoplasmic calcium concentration response [106] and phosphorylation of cytosolic proteins at tyrosine residues in these cells [53, 106]. These events appear to occur because FcR γ is up-regulated and may substitute for the deficient CD3 ζ chain and facilitate TCR/CD3 complex-mediated signaling [7, 22]. The increased expression of FcR γ observed in T lymphocytes from patients with SLE seems to confirm this hypothesis [7, 22]. Moreover, Enyedy et al. [22] demonstrated that FcR γ in peripheral blood T cells from patients with SLE participates in T cell signaling by associating with Syk kinase and CD3 ϵ . These findings may indicate that in T cells from patients with SLE, the switch in the TCR/CD3 signaling pathway, consisting of the change of the TCR/CD3 ϵ /CD3 ζ /ZAP-70 complex to a TCR/CD3 ϵ /FcR γ /Syk complex, exists and that these cells can represent effector CD4 $^{+}$ T cells generated as a result of chronic immune activation (see Fig. 3) [46].

The association between CD3 ζ chain and FcR γ in peripheral blood T cells from patients with SLE constitutes a very intriguing issue. In the light of recent studies indicating that increased expression of FcR γ results in down-regulation of the CD3 ζ chain in peripheral blood T cells [69], FcR γ may be one of the factors contributing to the reduction in CD3 ζ chain expression in T cells from patients with SLE. The positive correlation between increased expression of FcR γ in CD4 $^{+}$ T cells from patients with SLE and disease activity [7] and the fact that low levels of CD3 ζ chain correlate with increased expression of FcR γ in these cells [22] seem to confirm this possibility. On the other hand, most patients with SLE exhibit a sustained down-regulation of CD3 ζ chain expression over several months to years, although the disease activity fluctuates in some patients [72]. Considering the above, additional studies are required to understand the complementarity of the expressions of these two signaling molecules in peripheral blood T cells from patients with SLE.

In the following section we describe the factors and mechanisms that are responsible for the down-regulation of CD3 ζ chain expression in peripheral blood T cells from patients with SLE.

The factors and mechanisms responsible for the down-regulation of CD3 ζ chain expression and its abnormal function in T cells from patients with SLE

Results of biochemical and molecular studies indicate that several factors and mechanisms contribute to the reduction in CD3 ζ chain expression in peripheral blood T cells from patients with SLE. The first such pathological process is defective production of a functional 98-kDa form of E-74-like factor (Elf-1) observed

in these cells [36]. Elf-1, the transcription factor controlling CD3 ζ chain gene transcription, is present in the cytoplasm mainly in an 80-kDa form, and after phosphorylation and O-glycosylation it moves to the nucleus in a 98-kDa form which binds DNA [35]. Juang et al. [36] demonstrated that expression of the 98-kDa form of Elf-1 was either decreased or absent in T cells from patients with SLE. It has been also found that Elf-1 does not bind to the CD3 ζ chain gene promoter in T cells from patients with SLE, which consequently leads to decreased expression of CD3 ζ chain mRNA in these cells.

The next mechanism leading to decreased expression of CD3 ζ chain protein in peripheral blood T cells from patients with SLE are some alterations in the CD3 ζ chain mRNA open reading frame. The CD3 ζ chain gene spans at least 31 kb, and the transcript is generated as a spliced product of 8 exons that are separated by distances of 0.7 to more than 8 kb [34]. Nucleotide sequence analysis of the CD3 ζ chain mRNA in peripheral blood T cells from patients with SLE showed increased frequency of missense mutations in the coding region and alternatively spliced insertion and deletion forms compared with those from other rheumatic diseases and healthy controls [68, 103]. It is noteworthy that the missense mutations in the coding region and the spliced isoform were observed in cDNA and did not reflect the status of the genomic DNA variations. These results suggest defective mRNA editing and/or molecular misreading by defective RNA polymerase as possible mechanisms responsible for the CD3 ζ chain transcript mutations in peripheral blood T cells from patients with SLE [68]. A substantial number of these missense mutations were localized in ITAMs I, II, and III or the GTP/GDP binding domain of the CD3 ζ chain. These mutations could lead to functional alterations in the CD3 ζ chain and may be responsible for decreased expression of this protein. Indeed, immunoblot analysis using a CD3 ζ chain-specific monoclonal antibody showed that in the majority of the patients with coding-region missense mutations, the level of CD3 ζ protein was lower than in healthy subjects. In addition to the missense mutations in the coding region of CD3 ζ chain, there is a wide spectrum of alternatively spliced deletion and insertion isoforms of CD3 ζ chain mRNA in peripheral blood T cells from patients with SLE. Some of these alternatively splice variants are unique to T cells in patients with SLE [67, 68, 95]. The splice variant lacking exon 7 is the most common isoform of CD3 ζ chain mRNA occurring in peripheral blood T cells from patients with SLE [68]. What are the consequences of this deletion? First, translation of the exon 7-deleted isoform of CD3 ζ chain should produce a protein with an apparent molecular size of 14 kDa [68]. Indeed it has been reported that peripheral blood T cells from patients with SLE express a significantly higher amount of the 14-kDa form of CD3 ζ protein compared with those from controls [66]. Second, the 14-kDa form of CD3 ζ protein is functional-

ly altered following deletion of exon 7, since exon 7 spans the GTP/GDP binding site and the N-terminal tyrosine in the third ITAM domain of CD3 ζ chain [68, 95]. Thirdly, it has been found that the splice variant of CD3 ζ chain mRNA with deletion of exon 7 appeared to be less stable and more easily degraded than correct CD3 ζ chain mRNA [102]. Taken together, occurrence of the splice variant of CD3 ζ chain mRNA lacking exon 7 in peripheral blood T cells from patients with SLE leads to expression of a 14-kDa form of CD3 ζ protein that is functionally impaired, which contributes to altered signal transduction via the TCR/CD3 complex in these cells [102].

Another alternatively spliced variant of CD3 ζ chain mRNA occurring in peripheral blood T cells from patients with SLE is the variant with an alternatively spliced 3'-untranslated region (3'UTR). Nambiar et al. [67] and Tsuzaka et al. [101] demonstrated that T cells from patients with SLE predominantly generated CD3 ζ chain mRNA with the alternatively spliced 3'UTR, which is produced by a splice deletion of nucleotides from 672 to 1233 of exon 8 of the CD3 ζ chain transcript, whereas the expression of CD3 ζ chain mRNA with the wild-form 3'UTR was reduced. Moreover, the CD3 ζ protein expression level positively correlated with CD3 ζ chain mRNA with the wild-form 3'UTR and negatively with CD3 ζ chain mRNA with the short spliced variant of 3'UTR [101]. This correlation might suggest that the 562-bp region in 3'UTR of CD3 ζ chain mRNA is important for CD3 ζ protein expression and that T cells from patients with SLE whose CD3 ζ chain mRNA 3'UTR lacks this region may have low levels of CD3 ζ protein. Further studies seem to confirm this possibility. Tsuzaka et al. [99] and Chowdhury et al. [13] demonstrated that CD3 ζ chain mRNA with the short spliced variant of 3'UTR has lower stability than CD3 ζ chain mRNA with wild-form 3'UTR. What are the molecular mechanisms responsible for the destabilization of CD3 ζ chain mRNA with the alternatively spliced 3'UTR? Recently, Chowdhury et al. [12] identified two adenosine-uridine-rich elements within the 562-bp splice-deleted region in 3'UTR of CD3 ζ chain mRNA that are directly involved in the positive regulation of the transcription, mRNA stability, and sufficient translation of CD3 ζ chain mRNA. When these sequences (at positions 705 and 985) are absent, the CD3 ζ chain mRNA becomes unstable and poorly translated. Therefore the preferential production of the alternatively spliced forms of CD3 ζ chain mRNA lacking these critical sequences in its 3'UTR in T cells from patients with SLE represents a new, important mechanism that contributes to decreased expression of CD3 ζ chain protein in these cells [12, 13].

It is noteworthy that the predominant expression of the alternatively spliced variants of CD3 ζ mRNA (CD3 ζ mRNA lacking exon 7 and CD3 ζ mRNA with the alternatively spliced 3'UTR) in T cells from patients with SLE, which are less stable than the wild-form of

CD3 ζ mRNA, leads not only to the down-regulation of CD3 ζ chain protein expression, but also to the reduction in the expression of the TCR/CD3 complex on the cell surface [99, 102], since CD3 ζ chain is required for TCR/CD3 receptor assembly and its transport to the cell surface [26].

The next mechanism that may be involved in the reduction in CD3 ζ protein in peripheral blood T cells from patients with SLE is enhanced degradation of this molecule. In eukaryotic cells there are two distinct pathways for protein degradation: by lysosomal proteolytic enzyme within the lysosomal compartment or by the ubiquitin-proteasome pathway. In T cells from patients with SLE there seems to be enhanced degradation of the CD3 ζ molecule by lysosomal proteolytic enzyme as well as by the ubiquitin-proteasome pathway [6, 66]. Brundula et al. [6] observed increased degradation of CD3 ζ protein in the lysosomal compartment in T cells from patients with SLE. Moreover, the authors demonstrated that addition of NH₄Cl, a lysosomotropic agent which inhibits lysosomal enzyme activity, to *in vitro* cultured T cells restored CD3 ζ chain expression in these cells. In contrast, this treatment did not have a significant effect on CD3 ζ protein expression by T cells from healthy subjects. Nambiar et al. [66] found that the biochemical process of ubiquitination is dysregulated in T cells from patients with SLE, leading to an increased rate of ubiquitination and enhanced degradation by the proteasome pathway of the CD3 ζ protein in these cells. Recently, Krishnan et al. [47] proposed that increased expression and activity of caspase-3 observed in T cells from patients with SLE may be one of the processes responsible for the reduction in CD3 ζ chain protein in these cells. CD3 ζ protein bears several caspase-cleaving sites in its cytoplasmic domain [25], and caspase-3 may be involved in CD3 ζ chain digestion [25, 59]. Krishnan et al. [47] found that T cells from patients with SLE display higher levels and activity of caspase-3 than normal T cells. Treatment of T cells from patients with SLE with the caspase-3 inhibitor Z-Asp-Glu-Val-Asp-FMK reduced the proteolysis of CD3 ζ chain and increased the amounts of this protein in these cells.

In summary, the reduction in CD3 ζ protein in T cells from patients with SLE could result from the combined effect of several regulatory processes at the level of transcription, posttranscription, as well as protein degradation. However, on the strength of our current knowledge we cannot conclude which molecular events account for the induction of the processes leading to the down-regulation of CD3 ζ chain expression in peripheral blood T cells from patients with SLE.

A deficiency of CD3 ζ chain as one of the pathomechanisms leading to SLE?

The observations that CD3 ζ molecule is either absent or expressed at greatly decreased levels in peripheral blood T cells in the vast majority of patients with

SLE and that these defects are stable over a long period of time, regardless of the disease activity [6, 53, 72, 95, reviewed in 94], raise the question whether a deficiency of CD3 ζ chain in peripheral blood T cells from patients with SLE may be one of the factors in the pathogenesis of this disease. If this is true, normalization of CD3 ζ chain expression should restore T cells from patients with SLE to normal function. Nambiar et al. [70] showed that transfection of freshly isolated T cells from patients with SLE with a CD3 ζ chain construct results in an increase in the surface expression of the TCR/CD3 complex, normalization of the TCR/CD3-mediated phosphorylation of cellular protein substrates and the $[Ca^{2+}]$ response, augmentation of IL-2 production, and reduction in Fc γ R expression in these cells. These findings suggest that reconstitution of a deficient CD3 ζ chain can restore most of the phenotype of T cells from patients with SLE to normal. Thus, low levels of CD3 ζ chain play a substantial role in orchestrating T cell signaling abnormalities and T cell lymphokine production, and maintaining the expression levels of CD3 ζ chain is a vital component in the prevention of signaling aberrations [70].

Moreover, the results of current transfection and molecular studies may indicate that the CD3 ζ chain mRNA aberrations characteristic of patients with SLE result in alterations in the transcription of several genes encoding membrane proteins and TCR costimulatory molecules. Tsuzaka et al. [100] examined the gene expression profiles of T cell hybridoma MA5.8 cells expressing only either the splice variant of CD3 ζ chain mRNA lacking exon 7 or CD3 ζ chain mRNA with the short spliced variant of 3'UTR. The results showed that the genes encoding cytokines such as IL-2, IL-13, IL-15, IL-18, and transforming growth factor- β 2, chemokines (CCL3, CCL5, CCL9, XCL1), and a chemokine receptor (CCR7) were down-regulated in these cells. This observation may indicate that down-regulation of CD3 ζ chain can lead to aberrant signal transduction followed by reduced expressions of these cytokines and chemokines. Other genes were up-regulated in T cell hybridoma MA5.8 cells transfected with CD3 ζ chain mRNA splice-variant forms and included the genes encoding membranous proteins, such as syndecan-1 and poliovirus receptor-related 2, which are involved in cell-cell and cell-extracellular matrix interaction. Thus T cells with reduced expression of CD3 ζ chain may exhibit aberrant cell adhesion. It is noteworthy that up-regulated expression and function of adhesion molecules was observed in T cells from patients with active SLE [reviewed in 94]. Among the up-regulated genes in T cell hybridoma MA5.8 cells transfected with CD3 ζ chain mRNA splice-variant forms, the genes encoding cytotoxic molecules such as Gzma or MMP-11 were found. These proteins may be responsible for tissue damage [100]. Moreover, several molecules which account for lipid transport and metabolism, such as Pmm1, Lpn2, and Fads2, were also found to be increased in T cell hybridoma MA5.8 cells transfected

with CD3 ζ chain mRNA splice-variant forms. Although the above-described results are very interesting, altered expressions of the mentioned genes should be confirmed in T cells from patients with SLE.

In the light of these findings, a deficiency of CD3 ζ chain in peripheral blood T cells from patients with SLE may be one of the factors in the pathogenesis of this disease.

Another autoimmune disease in which the down-regulation of CD3 ζ chain expression was observed is RA.

RHEUMATOID ARTHRITIS

The role of effector CD4⁺ T cells in RA

RA is one of the most common autoimmune diseases, affecting about 0.5–1% of the population worldwide [reviewed in 105, 110]. This systemic disease is marked by chronic inflammation of synovial joints, which leads to progressive joint destruction and in many cases results in severe disability and poor quality of life. Although the precise cause of this disease is not fully clarified, it has been suggested that the inflammatory events in RA are initiated by CD4⁺ T cells recognizing antigens in the synovial tissue [reviewed in 108, 109]. Among the autoantibodies associated with RA, serum rheumatoid factor, type II collagen, and antibodies against proteins that are post-translationally modified by the enzymatic conversion of arginine to citrulline (anti-CP) were identified [11, reviewed in 79, 105, 110]. It has been proposed that autoreactive CD4⁺ T cells infiltrate the synovial membrane, where they initiate and maintain activation of macrophages and synovial fibroblast, transforming them into tissue-destructive effector cells [reviewed in 108, 109]. Indeed, the joint does not normally contain immune cells, but the synovial tissue of patients with RA is infiltrated by a variety of immunological cells, including T and B lymphocytes, macrophages, and neutrophils [reviewed in 32, 63, 110]. The role of CD4⁺ T cells in RA pathogenesis is supported by the strong association between the development of this disease and specific alleles encoded within the human leukocyte antigen (HLA)-DR region [reviewed in 16, 17, 32, 63, 110]. It has been found that people with the HLA-DRB1 allele, particularly with the subtypes DRB1*0401 (Dw4), DRB1*0404 and *0408 (Dw14), and DRB1*0405 (Dw15), are associated with susceptibility to development of RA [reviewed in 32, 110]. On the strength of current knowledge, the following model for a role of CD4⁺ T cells in the pathogenesis of RA is proposed (see Fig. 4) [reviewed in 16, 17, 63]. In the early phase of inflammatory responses, antigen-drive activation of CD4⁺ T cells and their differentiation to effector cells through the TCR/CD3 signaling complex ("antigen mode") predominates. In an unsusceptible host, the immune response resolves through mechanisms such as activation-induced cell death and/or the production of immunoregulatory cytokines. In a suscep-

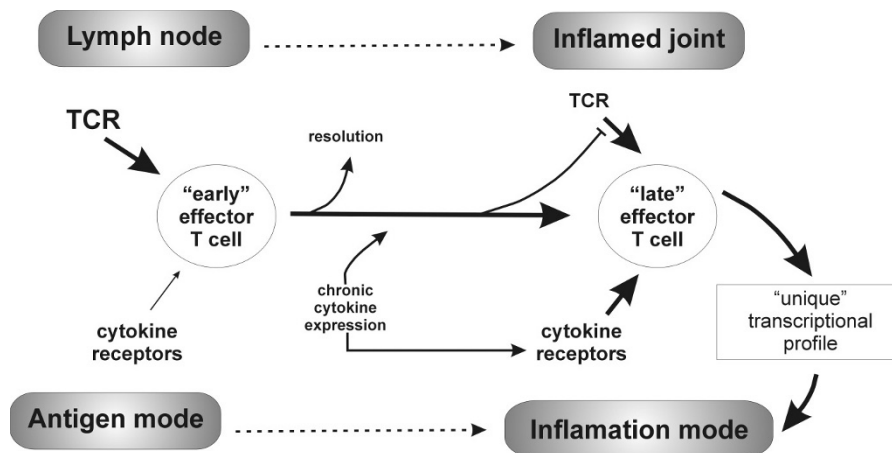


Fig. 4. A model for the role of CD4⁺ T cells in the pathogenesis of chronic inflammation, such as RA (according to Cope [17], copyright permission received from Oxford University Press for figure reproduction). The antigen-drive activation of CD4⁺ T cells and their differentiation to effector cells through the TCR/CD3 complex predominates during the early phase of inflammatory responses (“antigen mode”). In a susceptible host, additional CD4⁺ T cells are recruited to sites of inflammation. As the inflammatory process progresses, chronic cytokine production induces the hyporesponsiveness of CD4⁺ T cells to TCR/CD3 engagement. The TCR/CD3 hyporesponsive CD4⁺ T cells function as effector cells and sustain the chronic inflammatory process through a cytokine-driven signal-transduction pathway (“inflammation mode”).

tible host, additional CD4⁺ T cells are recruited to the site of inflammation through bystander activation or by stimulation with self antigens released from inflamed tissues. The conventional TCR/CD3-activated T lymphocytes are able to induce monocyte production of tumor necrosis factor (TNF)- α , consequently leading to an increase in the amount of this pro-inflammatory cytokine in the synovial fluid [reviewed in 63]. Indeed, in the synovial fluid of patients with RA, various pro-inflammatory cytokines, such as TNF- α and IL-1, have been detected [reviewed in 17, 63, 110]. The chronic exposure of T cells to inflammatory cytokines such as TNF- α induces a reversible, non-deletional hyporesponsiveness of T cells to TCR/CD3 engagement. In fact, T cells isolated from the synovial fluid have poor proliferative responses to a variety of stimuli [1, 54, 88] and produce decreased levels of IL-2 following activation [54]. The cross-linking of the TCR/CD3 complex induces a 2- to 3-fold lower Ca²⁺ influx response of synovial fluid T cells compared with controls [61], although they express increased levels of activation markers, including CD69, CD44, and HLA-DR, on their surface [29, reviewed in 32]. However, although these cells are TCR hyporesponsive, they are not inert, but have the potential to become potent effector cells through dominant cytokine-driven signal transduction pathways (“inflammation mode”) [reviewed in 16, 17, 63]. Synovial fluid T cells are responsive to pro-inflammatory cytokines present in the synovial microenvironment since they bear cognate cytokine receptors preferentially up-regulated on synovial compared with peripheral blood T cells [reviewed in 17, 32, 63]. In short, during the evolution of immune and inflammatory responses, the balance of stimulation shifts from “antigen mode”, in which T cells are engaged through the TCR/CD3

complex during the early phases of inflammatory responses, to “inflammation mode”, in which T cell activation and effector responses are driven by pro-inflammatory cytokines [reviewed in 16]. Cytokine-driven effector CD4⁺ T cells sustain the chronic inflammatory process by directing cytokine production, tissue factor, and matrix metalloproteinases to the site of chronic inflammation, leading to tissue destruction and progression of disease [reviewed in 63]. It is proposed that by reversing T cell hyporesponsiveness, antigen-dependent responses that serve to regulate the inflammatory process (e.g. through the expression of immunoregulatory cytokines) are restored [reviewed in 16, 17].

An intriguing question is whether in effector CD4⁺ T cells from patients with RA generated as a result of chronic immune activation there is a switch in the TCR/CD3 signaling pathway, consisting of a change in the TCR/CD3 ϵ /CD3 ζ /ZAP-70 complex to a TCR/CD3 ϵ /Fc γ R/Syk complex. Unfortunately, to the best of our knowledge, information concerning this phenomenon in these cells is lacking so far.

The molecular basis of synovial fluid T cell hyporesponsiveness to TCR/CD3 engagement and mechanisms leading to CD3 ζ chain down-regulation

What is the molecular basis of synovial fluid T cell hyporesponsiveness to TCR/CD3 ligation? An extensive series of studies revealed that signal transduction via the TCR/CD3 structure of synovial fluid T cells from patients with RA is defective [5, 57, 58, 61, 80]. It has been demonstrated that T cells isolated from the synovial fluid of patients with RA expressed lower levels of TCR $\alpha\beta$ [80], CD3 ϵ [57, 80], LAT [80], and CD3 ζ chain [5, 57, 58, 80] than peripheral blood T lymphocytes iso-

lated from patients with RA and healthy controls. It has been found that reduced level of CD3 ζ chain expression in synovial fluid T cells correlated with decreased proliferative response to stimulation with anti-CD3 antibodies [5, 58, 80]. Since CD3 ζ chain is required for TCR/CD3 receptor assembly and its transport to the cell surface [26], down-regulation of CD3 ζ chain expression in synovial T cells impairs TCR/CD3 receptor assembly and its expression at the cell surface, consequently leading to uncoupling of the membrane-proximal TCR/CD3 signaling pathway and hyporesponsiveness of these cells to TCR/CD3 engagement [33].

What are the mechanisms responsible for the reduction in CD3 ζ chain expression in synovial fluid T cells from patients with RA? The fact that down-regulation of this molecule's expression was observed above all in synovial fluid T cell, but not in peripheral blood T cells [5, 58, 80] (there is only one paper describing reduced CD3 ζ chain expression in peripheral blood T cells [57]), suggests that acquired, rather than inherited, factors would mainly account for the reduction in CD3 ζ chain expression. Thus a factor or factors present in the chronic inflammatory process in joints are involved in inducing CD3 ζ chain down-regulation [reviewed in 32]. One of these factors is TNF- α . It has been well documented that prolonged exposure of T cells to TNF- α leads to a reversible loss of CD3 ζ chain expression in these cells [33]. The precise molecular mechanisms responsible for the down-regulation of CD3 ζ chain expression by TNF- α are not clear. Isomäki et al. [33] suggested that TNF- α influences CD3 ζ chain expression at both the transcriptional and post-transcriptional levels, since it reduces CD3 ζ chain mRNA as well as CD3 ζ protein expression. The authors found that at higher concentrations, TNF- α down-regulates CD3 ζ mRNA, but at concentrations that have minimal effects on CD3 ζ mRNA expression, TNF- α reduces CD3 ζ protein level. Additionally, they showed that TNF- α down-regulates CD3 ζ protein indirectly by reducing intracellular glutathione levels, since N-acetylcysteine, a biosynthetic precursor of glutathione, inhibits the reduction of CD3 ζ protein expression induced by TNF- α . It is noteworthy that TNF- α down-regulates CD3 ζ chain expression but does not influence the expression of the CD3 γ , δ , and ϵ chains associated with the TCR/CD3 complex [33]. Therefore, TNF- α selectively down-regulates the expression of CD3 ζ chain and this leads to the disruption of TCR/CD3 receptor assembly and transport to the cell surface and to the uncoupling of both membrane-proximal and -distal signal transduction pathways.

What other factors or mechanisms may account for the reduction in the expression of CD3 ζ chain in synovial fluid T cells? It has been considered that macrophages and granulocytes may be involved in the decrease in CD3 ζ chain expression in patients with RA. Matsuda et al. [57] suggested that activated normal macrophages, present at a high frequency in the joints of patients with RA [reviewed in 63], may induce the reduction in CD3 ζ chain and other structural components of the CD3

complex via their production of free oxygen radicals. On the other hand, Berg et al. [5] demonstrated that granulocytes, but not macrophages, were able to down-regulate the expression of CD3 ζ chain.

In the light of our current knowledge, it seems that the down-regulation of CD3 ζ chain observed in synovial fluid T cells from patients with RA is not one of the pathomechanisms leading to RA, but is rather a consequence of the influence of the inflammatory microenvironment.

IDIOPATHIC NEPHROTIC SYNDROME IN CHILDHOOD

Idiopathic nephrotic syndrome (INS) in childhood, the most frequent glomerular disease in children, is a heterogeneous group of glomerular disorders and is characterized clinically by massive proteinuria, hypoalbuminemia, edema, and, frequently, hypercholesterolemia [reviewed in 2, 20, 30, 89, 104]. The development of the clinical changes typical of INS is closely correlated with specific structural changes in the foot processes of podocytes, which in part comprise the kidney's filtration barrier. Based on the response to steroid therapy, this disease is generally divided into steroid-sensitive, steroid-dependent, and steroid-resistant nephrotic syndrome. Most children with INS respond to steroid therapy, although they may be subject to more or less frequent relapses. In frequently relapsing patients, or in the case of steroid-dependent and steroid-resistant patients, prolonged remission can be obtained by using cyclosporin A, levamisole, cyclophosphamide, or chlorambucil [reviewed in 20, 23, 30, 104]. Although there have been extensive studies in humans as well as in a well-established rat model of nephrotic syndrome (puromycin aminonucleoside nephrosis), the precise cause of this disease remains unknown. In recent years it has been suggested that abnormalities of the immune system, and T-lymphocyte function in particular, are implicated in the pathogenesis of INS. Several immunological abnormalities observed in patients with INS, such as a markedly reduced response of T lymphocytes to stimulation by mitogens [27], increased concanavalin A-activated suppressor T cell activity [83], alterations in immunoglobulins synthesis [62], abnormal B cell function controlled by T cells [10, 40, 56, 113], abnormalities in the distribution of several lymphocyte subsets [19, 31, 41, 43, 51, 90, 97, 112], an imbalance of the circulating cytokines [19, 31, 51, 84, 90–92, 97, 113, reviewed in 3], and abnormalities in the intracellular production of cytokines [39, 56, 114, reviewed in 3], are in favor of this hypothesis. Furthermore, it appears that T cells in patients with INS release a certain circulating factor or factors (lymphokines, cytokines) that alter the glomerular permeability of the filtration barrier, contributing to proteinuria [reviewed in 4, 20, 104]. Although many efforts have been made to identify this pathogenic cytokine(s), its nature as well as the mechanism(s)

responsible for the release of this factor remain elusive. On the strength of current knowledge it has been hypothesized that some extrinsic agents, such as viral infection or allergens, or other environmental factors stimulate sensitized lymphocytes to produce a number of highly active cytokines, causing immunoregulatory imbalances that play a key role in the induction and development idiopathic non-familial nephrotic syndrome [reviewed in 104]. The fact that relapses are frequently triggered by viral infection or allergens seems to confirm this hypothesis. The evidence that lymphocyte dysfunction is strongly associated with INS has led to various studies attempting to identify the subset of lymphocytes playing an important role in the pathogenesis of this disease, but results have been conflicting. Recently it was proposed that suppressor/cytotoxic T cells (CD8⁺ T lymphocytes), natural killer (NK) cells, or monocyte/macrophage, but not helper/inducer T cells (CD4⁺ T lymphocytes), are involved in the course of INS [24, 39, 90]. Based on sequence analyses of the β -chain of complementarity-determining region 3 (CDR3) of the TCR receptor region, Frank et al. [24] suggested that in patients with INS there is oligoclonal expansion of CD8⁺ T cells. The CDR3 region of the TCR β chain is essential for peptide recognition [18], and deviations from the normal CDR3 length distribution reflect selected expansions of the corresponding T cells. This oligoclonal expansion of CD8⁺ cells has been described in several autoimmune diseases, such as RA [28] and SLE [44], and in viral infections [14, 21, 87]. Based on data obtained from CDR3 sequence analyses, Frank et al. [24] proposed that INS is driven by an autoimmune process or chronic infections.

To the best of our knowledge, papers concerning CD3 ζ chain expression in children with INS are lacking so far. Our preliminary studies indicated that the level of CD3 ζ chain expression in peripheral blood CD4⁺ T cells from children with INS in active phase is significantly higher than in patients in complete remission and in healthy children [65]. There were no differences between the levels of CD3 ζ chain expression in peripheral blood CD8⁺ T lymphocytes and NK cells from children in the active phase of INS, in complete remission, and in a control group. *Ex vivo* stimulation had no impact on CD3 ζ chain expression in patients with the active phase of INS, whereas in children in complete remission, stimulation with anti-CD3 monoclonal antibodies in the presence of rIL-2 increased the expression of this molecule in CD4⁺ T lymphocytes and decreased it in NK cells. Our preliminary results show alterations in CD3 ζ chain expression in children with INS. Further studies are needed to explain the molecular mechanisms responsible for this process.

CONCLUSIONS

The data summarized in this review show different patterns of CD3 ζ chain expression in T and NK cells in

patients with SLE, RA, and INS. The biochemical and molecular studies revealed that the factors and mechanisms responsible for altered expression and function of this signal-transducing molecule in these diseases are various. This observation is a considerable handicap in the creation of one universal model explaining alteration in CD3 ζ chain expression in human chronic inflammatory and autoimmune diseases. Further studies are required to elucidate these mechanisms in order to provide us with the necessary background information for generating therapeutic strategies in these diseases.

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