

TCR ζ -CHAIN DOWNREGULATION: CURTAILING AN EXCESSIVE INFLAMMATORY IMMUNE RESPONSE

Michal Baniyash

Abstract | The T-cell receptor (TCR) functions in both antigen recognition and signal transduction, which are crucial initial steps of antigen-specific immune responses. TCR integrity is vital for the induction of optimal and efficient immune responses, including the routine elimination of invading pathogens and the elimination of modified cells and molecules. Of the TCR subunits, the ζ -chain has a key role in receptor assembly, expression and signalling. Downregulation of TCR ζ -chain expression and impairment of T-cell function have been shown for T cells isolated from hosts with various chronic pathologies, including cancer, and autoimmune and infectious diseases. This review summarizes studies of the various pathologies that show this phenomenon and provides new insights into the mechanism responsible for downregulation of ζ -chain expression, its relevance and its clinical implications.

Immune responses directed against pathogens, tumour cells and modified self-antigens occur following the initial step of recognition of specific antigen and the transmission of activating signals, which are mediated by the T-cell receptor (TCR). Under normal conditions, activated T cells recruit and activate various cells of the adaptive and innate branches of the immune system, resulting in a controlled immune response. After antigen elimination, the responding T cells are 'turned off', and after a resting period, they are primed for re-activation following secondary antigenic stimulation.

During the past decade, various reports have been published showing that in cancer, infection and autoimmune disorders, which are all considered to be associated with chronic inflammation, T cells are functionally impaired. In all of these cases, it has been shown that the immune dysfunction is associated with loss of expression of the TCR ζ -chain. Interestingly, a bystander effect has also been noted, through which TCR ζ -chain expression is also downregulated by T cells that do not directly participate in eliciting the specific immune response. Consequently,

such hosts suffer from a generalized partial or severe immunodeficiency. Although many reports have described this phenomenon, few studies have attempted to delineate its immunological and molecular basis, and its clinical implications. These observations raise several unresolved and intriguing questions. Is the downregulation of ζ -chain expression an evolutionarily conserved escape mechanism that is induced by various pathologies or pathogens? What are the mechanisms that are responsible for the induction of downregulation of ζ -chain expression? Do the various pathologies have common factors that could be responsible for downregulation of ζ -chain expression? And how does downregulation of ζ -chain expression extend through a bystander effect to T cells that do not participate in the specific immune response? Answering these questions is of great importance, especially for understanding the functional characteristics of T cells in chronic inflammatory diseases and for designing various immunotherapeutic strategies to treat such diseases. In this review, some of these questions are addressed and their immunological and clinical implications are discussed.

The Lautenberg Center for
General and Tumor
Immunology, The Hebrew
University-Hadassah
Medical School, Post Office
Box 12272, Jerusalem
91120, Israel.
e-mail:
baniyash@cc.huji.ac.il
doi:10.1038/nri1434

TCR structure and signalling function

The TCR is a multisubunit complex (FIG. 1) that comprises at least six polypeptides. The clonotypic α - and β -chains are responsible for the recognition of antigen that is presented on the surface of antigen-presenting

cells (APCs) in the context of MHC molecules. The invariant subunits of CD3 form heterodimers, $CD3\gamma$ - $CD3\epsilon$ and $CD3\delta$ - $CD3\epsilon$, and together with the invariant ζ - ζ homodimer, they couple antigen recognition to intracellular signal-transduction pathways¹⁻⁴.

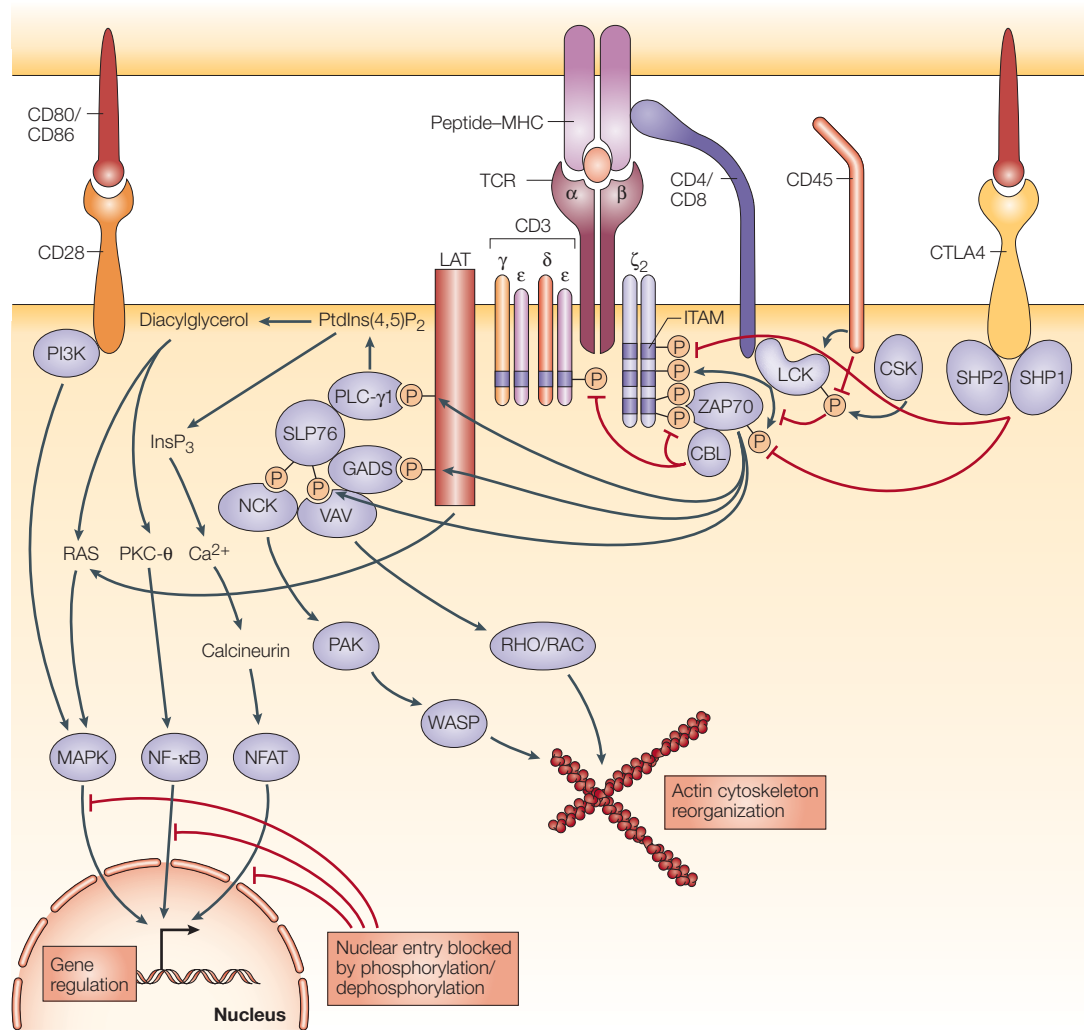


Figure 1 | Activation and attenuation signals controlling TCR-mediated T-cell function. Following T-cell receptor (TCR) and CD28 co-receptor engagement, SRC protein tyrosine kinases (PTKs) — LCK and FYN — are activated and phosphorylate the ζ - and ϵ -chains of the TCR at tyrosine residues that are present in ITAMs (immunoreceptor tyrosine-based activation motifs). Phosphorylated ITAMs in the ζ -chain function as docking sites for the recruitment of the PTK ZAP70 (ζ -chain-associated protein kinase of 70 kDa), which subsequently phosphorylates several substrates — LAT (linker for activation of T cells), SLP76 (SRC homology 2 (SH2)-domain-containing leukocyte protein of 76 kDa) and PLC- γ 1 (phospholipase C- γ 1). These phosphorylated substrates then recruit various catalytic and non-catalytic proteins — VAV, GADS (growth-factor-receptor-bound protein 2 (GRB2)-related adaptor protein) and NCK (non-catalytic region of tyrosine kinase). These proteins promote numerous intracellular signal-transduction pathways, leading to the activation of RAS, the mobilization of calcium ions (Ca^{2+}), the activation of protein kinase C (PKC) and calcineurin, and the polarization of the actin cytoskeleton. Subsequently, newly formed micro- and macromolecular structures facilitate the formation of the immunological synapse and the full activation of T cells. Complete activation of T cells is indicated by the following: activation of the transcription factors NF- κ B (nuclear factor- κ B) and NFAT (nuclear factor of activated T cells); transcription of cytokine genes; polar secretion of cytokines; proliferation of T cells; and recruitment of various cells of the adaptive and innate immune systems. Following T-cell activation, several attenuating mechanisms operate (shown in red), as a result of which the TCR is internalized, targeted for ubiquitination by CBL (Casitas B-lineage lymphoma) (with the involvement of ZAP70) and degraded mainly in the lysosome. Also, CTLA4 (cytotoxic T-lymphocyte-associated antigen 4) is expressed and becomes phosphorylated on inhibitory motifs that recruit the protein tyrosine phosphatases (PTPs) SHP1 (SH2-domain-containing PTP1) and SHP2, which in turn dephosphorylate the ζ -chain, protein kinases and other substrates. In parallel, entry of transcription factors to the nucleus is prevented by phosphorylation and dephosphorylation events. The PTP CD45 participates in both the activation and attenuation processes. CSK, carboxy-terminal SRC kinase; InsP₃, inositoltrisphosphate; MAPK, mitogen-activated protein kinase; PAK, p21-activating kinase; PI3K, phosphatidylinositol 3-kinase; PtdIns(4,5)P₂, phosphatidylinositol-4,5-bisphosphate; RHO, RAS homologue; WASP, Wiskott-Aldrich syndrome protein.

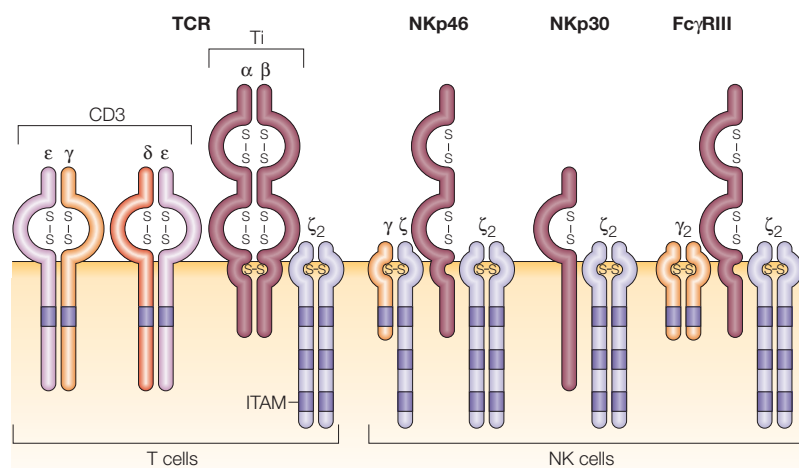


Figure 2 | ζ -chain structure and interactions in T cells and NK cells. A schematic representation of ζ -chain-containing receptor complexes. In T cells, the ζ -chain homodimer is part of the T-cell receptor (TCR) multisubunit complex. In natural killer (NK) cells, the ζ -chain forms a homodimer or a heterodimer with the γ -chain of the receptor for IgE (Fc ϵ R γ). This associates with two activating receptors, NK-cell protein 46 (NKp46) and NKp30, and the low-affinity Fc receptor for IgG (Fc γ RIII; also known as CD16), which mediates antibody-dependent cell-mediated cytotoxicity. The ζ -chain contains three ITAMs (immunoreceptor tyrosine-based activation motifs), which undergo tyrosine phosphorylation after engagement of each of these receptors and so have an important role in the activation process of both T cells and NK cells. T_H, TCR immune-recognition subunits.

CBL
A 120-kDa protein that functions as a RING-type E3 ubiquitin ligase. CBL targets the T-cell receptor–CD3 complex for ubiquitin conjugation, through the tyrosine kinase ZAP70 (ζ -chain-associated protein kinase of 70 kDa). In addition to proteasome- and lysosome-mediated degradation, ubiquitylation affects diverse biological processes, such as receptor downmodulation, signal transduction, protein processing and translocation, protein–protein interactions and gene transcription.

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs). Regions in the cytoplasmic domains of the invariant chains that are part of various cell-surface immune receptors, such as the T-cell receptor, the B-cell receptor, the receptor for IgE (Fc ϵ R) and natural-killer-cell activating receptors. Following phosphorylation, the ITAMs function as docking sites for SRC homology 2 (SH2)-domain-containing tyrosine kinases and adaptor molecules, thereby facilitating intracellular signalling cascades.

Assembly of the TCR subunits is a complex event that is tightly controlled; whereas complete TCR complexes are displayed on the cell surface, partially assembled TCRs are retained in the endoplasmic reticulum or targeted for degradation⁵. However, exceptions to this paradigm are observed in the pathologies associated with downregulation of ζ -chain expression that are discussed here; in these cases, despite the absence of the ζ -chain, TCRs are present on the T-cell surface at normal concentrations.

The general view of the immediate and late events in TCR signalling that occur after antigen–MHC recognition is shown in FIG. 1. These involve the formation of complex micro- and macromolecular structures on the cell surface and in the cell^{2,3,6,7}; these structures facilitate the sustained TCR-mediated signalling that is required for complete T-cell activation, as indicated by polar cytokine secretion, cellular proliferation, and the recruitment of various cells of the adaptive immune system (such as cytotoxic T cells (CTLs), T helper (T_H) cells and B cells) and the innate immune system (such as macrophages, dendritic cells (DCs) and natural killer (NK) cells). The combined effects of the two systems lead to the clearance of the pathogen or aberrant cells⁸.

After the T-cell response is well established, it must also be carefully regulated and, eventually, terminated. T cells have an array of shut-down mechanisms that operate through diverse pathways, one of which involves the control of TCR expression. Following TCR-mediated activation, the entire TCR complex is internalized and all of its subunits are degraded in the lysosome^{9–11}. Although by 24 hours after the initial activation, new TCR chains are synthesized and intracellular levels return to normal, only low levels of TCR are present at

the cell surface¹¹. At this stage, the cells remain unresponsive to new antigenic stimuli for 72 hours or longer^{11,12}. Additional shut-down mechanisms also operate in T cells (FIG. 1); these involve, among others, the inhibitory co-receptor cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) (REF. 13), the E3 ubiquitin ligase CBL (Casitas B-lineage lymphoma)¹⁴, the protein tyrosine kinase (PTK) carboxy (C)-terminal SRC kinase (CSK) and the phosphatase calcineurin^{1–4}.

These complex TCR-mediated signalling events and the tight control mechanisms that fine-tune T-cell function are crucial for the execution of a balanced immune response. Any aberration in the structure or assembly of a TCR could potentially affect its function and lead to partial or severe immunodeficiency (depending on which molecule is affected), as has been shown for T cells that are isolated from hosts with cancer, or autoimmune or infectious diseases.

Properties of the TCR ζ -chain

The ζ -chain was first discovered in T cells^{15,16} as a component of the TCR, and later, it was also found to be a component of the activating receptors NK-cell protein 46 (NKp46), NKp30 and the low-affinity Fc receptor for IgG (Fc γ RIII; also known as CD16), which are expressed by NK cells^{17,18} (FIG. 2). Under normal conditions, in both T cells and NK cells, the ζ -chain has an important role in the expression and signalling function of these receptors. The ζ -chain differs from other TCR subunits in its genetic organization, chromosomal localization and protein structure^{16,19}. All of the TCR chains, except the ζ -chain, are members of the immunoglobulin-gene superfamily. The CD3 γ , δ and ϵ chains are structurally related and have significant sequence homology, and the genes that encode these subunits are clustered in 300 kilobases on human chromosome 11 and mouse chromosome 9. By contrast, the ζ -chain has no sequence or structural homology to the CD3 components, and the gene encoding the ζ -chain is localized to the distal part of chromosome 1 in a linkage group that is highly conserved between humans and mice. The ζ -chain is a 16-kDa transmembrane protein that is expressed by T cells and NK cells as a disulphide-linked homodimer. It is composed of a short extracellular domain (9 amino acids) followed by a long intracellular domain (113 amino acids) that contains three IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIFS (ITAMs), which undergo tyrosine phosphorylation during activation. Phosphorylation of the ζ -chain increases its apparent molecular weight to 21- and 23-kDa intermediates, and it enables the ζ -chain to bind and recruit various catalytic and non-catalytic molecules. Therefore, the ζ -chain is indispensable for coupling antigen recognition by the TCR to diverse signal-transduction pathways^{1–4,20} and for coupling antigen recognition by NK cells to lytic function. For example, my laboratory and other research groups have shown that in resting and activated T cells, the ζ -chain links ~30% of the cell-surface-expressed TCRs to the actin-based cytoskeleton^{21,22}. In addition, it has been suggested that the ζ -chain stabilizes the TCR on the cell surface and functions to maintain cell-surface

receptor expression by sterically blocking internalization sequences on other TCR components²³. In mature T cells, the ζ -chain is considered to be a limiting factor in the assembly of complete receptors and their successful transport to the cell surface, thereby dictating the number of TCRs that are displayed at the cell-surface and the functionality of these receptors^{1–4,9–11,24}. It has also been suggested that the 21-kDa phosphorylated form of the ζ -chain controls T-cell survival²⁵. This idea is based on studies showing that naive CD4⁺ T cells express a basal level of the 21-kDa phosphorylated form of the ζ -chain, which results from constitutive, weak MHC–TCR interactions. This 21-kDa phosphorylated form does not transmit a full activation signal, unlike the 23-kDa form, but it suffices for eliciting partial downstream events that ultimately promote survival.

This article focuses on studies that have been published in the past decade that indicate the possible role of the ζ -chain as a ‘sensor’ of sustained exposure to chronic inflammatory immune responses, which are typical of those generated in hosts with tumours, infections or autoimmune disorders, thereby clearly affecting the mode and magnitude of T-cell responses. This unique property of the ζ -chain has been indicated by various studies that show that of all of the TCR subunits, only expression of the ζ -chain is specifically downregulated in different chronic pathologies and also that these T cells have impaired TCR-mediated functions (TABLE 1). In many of these pathologies, similar alterations that are associated with downregulation of ζ -chain expression were also observed in NK-cell killing (TABLE 1).

Impaired ζ -chain expression and T-cell function

Both downregulation of ζ -chain expression and impairment of T-cell function have been reported in various pathologies that differ in their aetiology and physiology (TABLE 1). The early reports of downregulation of ζ -chain expression involved hosts with tumours^{26–47}, and in recent years, this finding has been extended to hosts suffering from autoimmune^{48–52} and infectious^{53–56} diseases.

Hosts with tumours. The first report to describe this phenomenon²⁶ showed that animals with the experimental colon carcinoma MCA38 have CD8⁺ T cells with impaired cytotoxic function. Moreover, in these animals, all T cells (both CD4⁺ and CD8⁺) have cell-surface-expressed TCRs that lack the ζ -chain. The authors also showed that the γ -chain of the receptor for IgE (Fc ϵ R γ) replaces the missing ζ -chain. Fc ϵ R γ and the ζ -chain have structural and functional similarities: Fc ϵ R γ contains one ITAM, has a protein structure that is similar to the ζ -chain and is present as a homodimer that assembles together with the TCR. TCRs that contain Fc ϵ R γ are displayed at the cell surface but have partial signalling function compared with TCRs that contain the ζ -chain homodimer. In this model system, the T cells have additional aberrations, such as low expression levels of the CD3 γ -chain and decreased expression of tumour-necrosis factor (TNF), granzyme B, and the PTKs LCK and FYN. T cells isolated from these animals were also reduced in their ability to mediate an antitumour response *in vivo*.

Table 1 | ζ -chain expression and cell dysfunction in chronic pathologies

Pathologies	Reduced ζ -chain expression (T cells)	Reduced ζ -chain expression (NK cells)	Cell dysfunction (T cells)	Cell dysfunction (NK cells)	Chronic inflammation	References
Cancers						
Colon	Yes	Yes	Yes	Yes	Yes	26–28,71
Renal	Yes	ND	Yes	ND	ND	29,30
Ovarian	Yes	Yes	Yes	Yes	Yes	31,32,72
Cervical	Yes	Yes	Yes	Yes	Yes	33,73
Breast	Yes	ND	Yes	ND	Yes	34,35,74,82
Prostate	Yes	Yes	Yes	ND	Yes	36,75
Head and neck	Yes	Yes	ND	ND	ND	37
Melanoma	Yes	Yes	Yes	ND	ND	38,39
Leukaemia	Yes	ND	ND	ND	ND	40,41
B-cell lymphoma	Yes	ND	Yes	ND	ND	42
Hodgkin's lymphoma	Yes	Yes	Yes	ND	ND	43
Pancreatic	Yes	ND	ND	ND	Yes	44,76
Autoimmune disorders						
Systemic lupus erythematosus	Yes	ND	Yes	ND	Yes	48,49,77
Rheumatoid arthritis	Yes	ND	Yes	ND	Yes	50–52,78
Infectious diseases						
HIV	Yes	Yes	Yes	Yes	ND	53,55
Leprosy	Yes	ND	Yes	ND	Yes	56,115

ND, not determined; NK, natural killer.

The authors suggested that these changes could be the basis of the immune defects that were observed in tumour-bearing hosts. Since then, many additional reports have been published that show aberrations in T cells isolated from hosts (both human patients and mice) with various solid and non-solid tumours^{26–30,32–44} (TABLE 1). In all of these reports, the most pronounced and reproducible observation was the total or partial loss of TCR ζ -chain expression, which correlated with impaired T-cell function *in vitro*. Aberrations in the expression or function of other signalling molecules, cytokines or transcription factors were not consistently observed. During the initial stages of growth of many of these tumours, downregulation of ζ -chain expression and impairment of T-cell function were detected in tumour-infiltrating lymphocytes; at later stages (usually following tumour progression), this phenomenon was also observed in peripheral-blood T cells. Several reports have also shown downregulation of ζ -chain expression by NK cells that were isolated from tumour-bearing hosts, and this correlated with impaired lytic function (TABLE 1). So, both the adaptive and innate immune systems are affected by the conditions that are generated in tumour-bearing hosts.

It has been suggested that downregulation of ζ -chain expression is one of the many escape strategies that have been developed by various malignancies to evade immune surveillance⁴⁵. This idea is based on several studies showing that factors secreted by tumours are responsible for the immunosuppression that results from downregulation of ζ -chain expression and abnormal T-cell function^{39,46,47}. Whether this is indeed the case is discussed later.

Hosts with autoimmune diseases. As research into disease-mediated immune suppression progressed, various studies analysing T cells and NK cells in autoimmune diseases noted the downregulation of ζ -chain expression and the impairment of T-cell and NK-cell function, similar to that observed in tumour-bearing hosts (TABLE 1). In the T cells of patients with SYSTEMIC LUPUS ERYTHEMATOSUS (SLE), it was observed that the levels of ζ -chain expression are low and that the Fc ϵ R γ chain is overexpressed and replaces the ζ -chain^{48,49}. Decreased levels of nuclear factor- κ B (NF- κ B) p65 (also known as REL-A), which is responsible for inducible NF- κ B activity after TCR stimulation, were also observed in the T cells of patients with SLE^{48,49}. For patients with rheumatoid arthritis, T cells isolated from the SYNOVIAL FLUID were found to have decreased expression levels of ζ -chain and impaired proliferation after mitogenic or antigenic stimulation, when compared with peripheral T cells, which showed normal characteristics. After stimulation of synovial-fluid T cells through TCR and CD3, tyrosine phosphorylation of the ζ -chain, which is one of the initial events in TCR signalling, was reduced, as was the early increase in intracellular calcium ions that is induced after activation^{50–52}.

A recent study in SKG mice⁵⁷, which spontaneously develop arthritis, has identified a spontaneous point mutation in the C-terminal SRC homology 2 (SH2)

domain of the PTK ZAP70 (ζ -chain-associated protein kinase of 70 kDa). This mutation causes a chronic autoimmune arthritis that resembles human rheumatoid arthritis in many respects. It was therefore suggested that altered signal transduction from the TCR through the aberrant ZAP70 affects the thresholds of T cells during thymic selection, leading to the positive selection of otherwise negatively selected autoimmune T cells⁵⁷. However, such a defect has not been described for other mouse models of rheumatoid arthritis or for patients, and of the various signalling molecules that have been tested, downregulation of ζ -chain expression is the most consistent defect observed in mouse and human rheumatoid arthritis. As a result, in hosts with these autoimmune disorders, T cells become dysfunctional during disease progression. However, it is difficult to understand how such immune dysfunction can co-exist with the aberrant and excessive antiself immune response that is usually associated with autoimmunity. This issue is discussed later.

Hosts with infectious diseases. Loss of ζ -chain expression and dysfunction of T cells have also been reported in various infectious diseases (TABLE 1). Individuals who are infected with HIV (but have not yet developed AIDS) usually have a high frequency of HIV-specific CD8⁺ T cells that lack detectable HIV-specific cytotoxicity and so are unable to control viral replication. These HIV-positive individuals have mild to advanced immunodeficiency, and a substantial proportion of their circulating T cells (both CD4⁺ and CD8⁺) have downregulated ζ -chain expression^{53,54}. Downregulation of ζ -chain expression was found in T cells that were isolated from patients at various stages of infection, and this reduction in expression correlated with impaired T-cell function. Additional defects, such as a marked decrease in the levels of expression of the PTKs LCK, FYN and ZAP70, were also observed in these patients. An impairment of HIV-specific CD4⁺ T_H cells, which would normally provide specific CD8⁺ T-cell stimulation, compromises the antiviral function of CTLs *in vivo* in most HIV-infected individuals^{53–55}. NK cells from HIV-infected patients are also affected, expressing considerably lower levels of the ζ -chain as the disease progresses. The CD16-dependent cytolysis that is mediated by NK cells is also impaired, possibly because of decreased ζ -chain expression⁵⁵.

Another infectious disease that is associated with downmodulation of ζ -chain expression is leprosy, which is caused by infection with *Mycobacterium leprae*. Advanced stages of mycobacterial diseases, such as leprosy and tuberculosis, are characterized by a loss of T-cell function. The basis of this T-cell dysfunction is most probably a reduction in ζ -chain expression⁵⁶. In some cases, the expression of LCK and NF- κ B p65 is also downregulated⁵⁶.

In summary, under the conditions that are generated by the various pathologies described, of all of the TCR subunits and signalling molecules that have been studied, only ζ -chain expression is consistently downregulated. In most cases, this downregulation correlates

SYSTEMIC LUPUS

ERYTHEMATOSUS

(SLE). An autoimmune disease in which autoantibodies specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, especially in the kidney. Despite extensive study, this disease is still not fully understood and differs from other autoimmune diseases in several respects.

SYNOVIAL FLUID

The fluid that accumulates in the joints of patients with rheumatoid arthritis, which is a common inflammatory joint disease that has a strong autoimmune component.

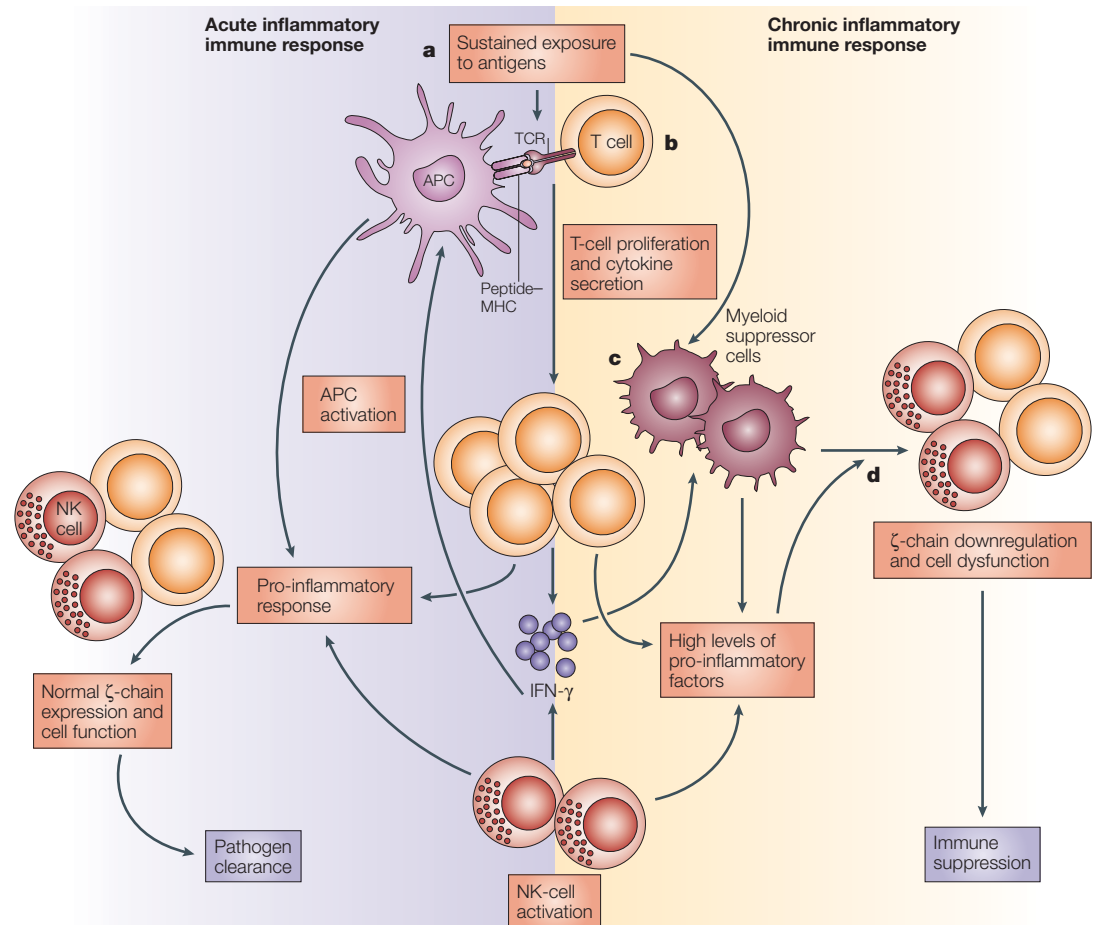


Figure 3 | ζ -chain expression in acute and chronic inflammation. Exposure to antigens (a) that induce T helper 1 (T_H1)-cell-dependent responses could lead to either acute or chronic inflammation, depending on the duration of antigen exposure. Following engagement of TCRs by antigen–MHC complexes displayed on antigen-presenting cells (APCs), antigen-specific T_H cells are activated (b), as indicated by their secretion of cytokines, their proliferation, and the activation of APCs and natural killer (NK) cells. Activation of various immune cells could also be mediated through the interaction of Toll-like receptors with pathogen-associated molecular patterns. In all of these situations, activated cells (T cells, APCs and NK cells) produce pro-inflammatory factors (including nitric oxide, hydrogen peroxide and prostaglandins) and cytokines (such as interferon- γ , IFN- γ) and tumour-necrosis factor), which result in the generation of an inflammatory response and thereby enable clearance of the pathogen. During an acute inflammatory response (left panel), all T cells and NK cells show normal levels of ζ -chain expression and normal cell function. By contrast, a chronic inflammatory immune response (right panel) results in a persistent inflammatory environment, as is observed in hosts with cancer, and infectious and autoimmune diseases. Increased levels of IFN- γ , together with antigen persistence, lead to the recruitment of numerous myeloid suppressor cells to the affected tissue and lymphatic organs (c). The continuous presence of myeloid suppressor cells and the increased levels of cytokines and other factors secreted by these cells negatively affect all surrounding T cells and NK cells, leading to their hyporesponsiveness and loss of ζ -chain expression (d). So, chronic inflammation prevents T-cell recovery and contributes to an overall immune suppression. TCR, T-cell receptor.

with impaired T-cell function and is observed in both T cells that are specific for the eliciting antigen and T cells that are not. However, in such affected T cells, even though expression of the ζ -chain is at a low level or absent, normal levels of TCR are observed at the cell surface. This is in contrast to TCR expression by normal T cells. In the absence of the ζ -chain in normal T cells, cryptic TCRs are assembled in the cells and targeted for lysosomal degradation, rather than targeted to the cell surface^{5,24}. It has been shown that in some hosts with tumours or SLE, Fc ϵ R γ expression can replace ζ -chain expression and lead to normal concentrations of cell-surface TCR — although these substituted TCRs still have impaired function after

antigen engagement^{26,58}. It is important to note that in most of the studies that describe downregulation of ζ -chain expression, Fc ϵ R γ was not detected as part of the TCR. So, two models could explain normal levels of cell-surface TCR in the absence of the ζ -chain: either a small amount of expressed ζ -chain functions as a chaperone and transports the assembled TCRs to the cell surface, and/or an as-yet-unidentified protein (not Fc ϵ R γ) is expressed under these pathological conditions and replaces the ζ -chain. Therefore, downregulation of ζ -chain expression can affect both adaptive (T cell) and innate (NK cell) immune responses, leading to partial or total immunosuppression under pathological conditions.

MYELOID SUPPRESSOR CELLS (MSCs). A population of cells composed of mature and immature myeloid cells. They are generated and/or activated during an inflammatory immune response. Through direct interactions and secreted components, they negatively affect T cells, which leads to the downregulation of ζ -chain expression and impairment of T-cell function.

Immunological basis for ζ -chain downregulation

To determine the immunological basis for downregulation of ζ -chain expression, my research group searched for factors common to the pathologies that are associated with this phenomenon. Comparison of the various immunological aspects of these pathologies led us to propose that sustained exposure to antigen and development of a chronic inflammatory response are most probably responsible for the induction of this phenomenon. Inflammatory responses are initiated locally but become systemic as disease progresses.

To test this hypothesis, an *in vivo* experimental system that mimics chronic inflammatory immune activation was set up in my laboratory⁵⁹. In this system, normal mice receive a series of injections of heat-killed Gram-negative bacteria, thereby providing sustained exposure to antigen; the bacterial antigens used are known to elicit an inflammatory immune response. Under such conditions, downregulation of ζ -chain expression and impairment of T-cell function are induced, provided that a T_H1 -cell-dependent inflammatory immune response develops. In this system, the immunosuppressive conditions that are generated are similar to those observed in the various pathologies: all types of T cell, not only those responsive to the specific antigen, are affected (which is known as the bystander effect); of all of the TCR subunits, only expression of the ζ -chain is downregulated; TCR concentrations at the cell surface are normal; and T-cell function (mediated by $CD4^+$ and $CD8^+$ cells) is impaired both *in vitro* and *in vivo*. Moreover, using gene-knockout mice, my research group also showed that interferon- γ (IFN- γ) secreted by activated T cells, APCs

and NK cells has an important role in the induction of the immunosuppressive environment, most probably by inducing the recruitment and/or generation of non-lymphoid cells (that is, GRI^+MAC1^+ myeloid cells) that induce downregulation of ζ -chain expression and impairment of T-cell function (REF. 59 and Ezernitchi, A., Vaknin, I., Cohen-Daniel, L. and M.B., unpublished observations) (FIG. 3). A similar population of immature myeloid cells with immunosuppressive function, known as MYELOID SUPPRESSOR CELLS (MSCs) (BOX 1), has been found in various tumour-bearing hosts⁶⁰ and during acute infection⁶¹. I suggest that these cells are responsible for the immunosuppressive environment that induces the bystander effect of downregulation of ζ -chain expression and impairment of T-cell function (FIG. 3). It is important to note that this phenomenon can be induced by various antigens that elicit an inflammatory response⁵⁹. Moreover, this phenomenon is reversible. Following withdrawal of an antigen, T cells recover, and ζ -chain expression levels and TCR-mediated functions are restored.

Although the proposed model of chronic antigen stimulation causing downregulation of ζ -chain expression has only been tested in infectious-disease settings, there is supporting evidence for this model in other situations. Numerous studies have reported the presence of specific antigens and the development of chronic inflammatory processes that are directly associated with disease progression. The antigens could be specifically expressed by the pathogen, tumour or self-tissues^{61–68}, thereby activating T cells that express specific TCRs. Antigens could also be related to the group of pathogen-associated molecular patterns (PAMPs) that activate Toll-like receptors (TLRs) (that is, TLR LIGANDS). TLRs and their various ligands have an important role in the excessive activation of MSCs^{69,70}. So, although some tumours lack specific antigens, they could nevertheless excessively and chronically activate the immune system through TLRs. Any of these antigen- or ligand-dependent modes of activation might induce acute or chronic inflammation. Indeed, many of these pathologies (TABLE 1) manifest a developing chronic inflammation that is directly associated with disease progression^{71–79}. Moreover, it has also been suggested that local or systemic chronic inflammation is a risk factor for the development of cancer and persistent infection, owing to the developing immunosuppression that accompanies disease progression^{80–83}. However, the general applicability of this model of chronic antigen stimulation requires further testing, and in some cases, other possible explanations for the downregulation of ζ -chain expression, such as the secretion of suppressive factors by tumour cells, cannot be ruled out.

The link between chronic inflammation, immunosuppression and the progression of tumours and infections is clear. However, it is more difficult to understand the relationship between immunosuppression and autoimmune disorders, which are thought to be initiated and mediated mainly by persistently activated T cells. If these T cells downregulate their ζ -chain expression and become hyporesponsive

Box 1 | Myeloid suppressor cells

In a developing inflammatory environment, the accumulation of regulatory $CD11b^+GRI^+$ myeloid suppressor cells (MSCs) is observed. This population is heterogeneous, and it has been detected in lymphoid organs during tumour growth, in graft-versus-host reactions and in infectious diseases⁶⁰. In all cases, impaired T-cell responses to T-cell receptor (TCR)-mediated stimuli are observed. MSCs have an important role in the regulation of the inflammatory process and in the control of T-cell responses. One of the mechanisms that is used by MSCs to control T cells is based on arginine metabolism. MSCs use two enzymes to metabolize arginine: inducible nitric-oxide synthase, which generates nitric oxide, and arginase-1, which converts arginine to urea and L-ornithine. Nitric oxide inhibits T-cell proliferation¹¹⁹, most probably by preventing activated T cells from entering the cell cycle, and L-ornithine, which is consumed by MSCs, is not available for the T cells that require it for cell proliferation and differentiation¹²⁰. Moreover, it was recently shown that L-arginine is required for expression of the ζ -chain of the TCR, through an as-yet-unknown mechanism⁸⁹. *In vitro* addition of excess L-arginine leads to re-expression of the ζ -chain and recovery of T-cell proliferation. Therefore, the regulation of L-arginine concentrations in the microenvironment could interfere with early signalling pathways and, consequently, could modulate T-cell function. So, MSC recruitment can have an important role in the control of excessive immune responses. The expansion of MSC populations in the lymphoid organs of infected or immunized mice is transient. By contrast, under conditions of chronic inflammation, such as during tumour progression and chronic infection, the number of MSCs remains high, and immunosuppression is maintained. MSCs suppress the activation of both $CD4^+$ and $CD8^+$ T cells in an antigen- and MHC-independent manner. Both cell–cell contact between MSCs and T cells, and secreted compounds, are required for the inhibitory activity of MSCs¹²¹. So, interference with the mechanisms used by MSCs to suppress T-cell function seems to be the most promising therapeutic strategy for restoring T-cell function.

TLR LIGANDS

A group of pathogen-associated molecular patterns — such as lipopolysaccharide, CpG-motif-containing DNA, double-stranded RNA and flagellin — that activate Toll-like receptors (TLRs). TLRs are expressed mainly by cells of the innate immune system (such as macrophages, dendritic cells and natural killer cells) and are also expressed by some cells of the adaptive immune system (T cells and B cells). Endogenous mammalian proteins — such as heat-shock proteins, DNA and extracellular-matrix components — which are characteristic of damaged tissues and typical of necrotic tumours, metastases, autoimmune diseases and infections, could also activate TLRs. TLRs and their various ligands have an important role in the excessive generation and/or activation of myeloid suppressor cells and in the induction of a chronic inflammatory immune response.

during a developing autoimmune disease, then how does the disease proceed? I suggest that during the development of an autoimmune response, the immune system of the host is excessively activated by autoantigen(s) and, at a later stage, by endogenous TLR ligands that activate various cells of the innate system. Tissue damage is caused by the activated cells of the adaptive immune system (T cells and B cells) and innate immune system (macrophages, DCs, NK and natural killer T (NKT) cells) that are recruited to the inflammatory site and their products (such as antibodies, cytokines, chemokines and oxidative factors). In turn, this leads to the development of a chronic inflammatory immune response, resulting in the downregulation of ζ -chain expression and the attenuation of T-cell function. In some autoimmune diseases, the immune system could recover; T cells would then regain their function and/or new T cells would arrive at the site of inflammation, and the activation–destruction cycle would resume. Indeed, such cycles of exacerbation and remission are characteristic of several autoimmune disorders, including multiple sclerosis and rheumatoid arthritis. So, it can be predicted that cycles of ζ -chain expression levels would be observed during the course of these diseases. However, the precise kinetics of ζ -chain expression in such disease cycles have not been examined. Recently, it has been shown that in patients with SLE, despite the fluctuating disease activity, the downregulation of ζ -chain expression is stable during the disease course⁸⁴. This stable downregulation of ζ -chain expression could result from the combined effect of several regulatory mechanisms that control ζ -chain expression in the T cells of patients with SLE and/or the continuous presence of immunosuppressive factors,

preventing cyclical recovery of ζ -chain expression. In the latter case, it is predicted that tissue damage is initiated by specific T cells (adaptive immune system) but is later maintained by the activated MSCs and their products (innate immune system). This hypothesis has yet to be tested.

The paradigm for the generation of an immunosuppressive environment that leads to downregulation of ζ -chain expression and dysfunction of T cells, whether triggered by infection, cancer or autoimmunity, is that there is a background of a chronic inflammatory immune response (BOX 2). So, it can be suggested that in the case of acute inflammation, the transient downregulation of ζ -chain expression and unresponsiveness of T cells that ensue are beneficial processes that help to attenuate an unbalanced and 'super-activated' immune system. By contrast, under conditions of chronic inflammation, long lasting downregulation of ζ -chain expression occurs together with impaired T-cell function that is characterized by partial or severe immunodeficiency, making this phenomenon detrimental to the host (BOX 2). The beneficial versus harmful effects of downregulation of ζ -chain expression and impairment of T-cell function that are described here are speculative and remain to be directly tested *in vivo*.

Mechanisms involved in ζ -chain downregulation

In a developing inflammatory environment, it seems that various cells of the myeloid lineage accumulate and induce downregulation of ζ -chain expression and dysfunction of T cells. So, there are two participants in the induced immunosuppression: the immunosuppressive environment — including its cells and secreted factors, which can affect both antigen-specific and non-antigen-specific T cells in the local area — and the affected T cells.

Box 2 | Teleological explanation for ζ -chain downregulation

What then is the physiological and immunological 'rationale' for the downregulation of ζ -chain expression and impairment of T-cell function that is observed in various chronic inflammatory conditions? Because this phenomenon is common to many different pathologies, it can be suggested that it is the normal outcome of an excessive and potentially hazardous inflammatory immune response. I propose (FIG. 3) that downregulation of ζ -chain expression starts with an 'innocent' immune response. Following antigen recognition, the activated T cells secrete specific cytokines, proliferate, become effector and memory cells and, in turn, further activate antigen-presenting cells (APCs). In addition, APCs could be activated through their Toll-like receptors by pathogen-associated molecular patterns^{69,70}. Over time, these stimuli generate an inflammatory response. The pathogen is then cleared, and the antigenic stimulus disappears; the inflammatory environment is also reduced, and the system returns to a resting state. Until this stage, the developing immune response is antigen driven and transient. However, if antigen is continuously present and the inflammatory environment persists, an antigen-independent process commences, which inactivates all T cells, thereby leading to T-cell hyporesponsiveness and loss of ζ -chain expression.

It can be suggested that in the case of a time-limited exposure to antigen, as occurs in acute bacterial or viral infection, the transient downregulation of ζ -chain expression and T-cell unresponsiveness that ensue might help attenuate the response and restore the balance of a 'super-activated' immune system. Indeed, in the model set up by my research group⁵⁹, mice regained normal ζ -chain expression and T-cell function within 10 days of the previous antigen injection. By contrast, in pathological conditions that show downregulation of ζ -chain expression, the continuous presence of antigen and the developing chronic inflammation most probably prevent recovery, thereby contributing to the pathological nature of the disease.

The immunosuppressive environment. Mature macrophages and regulatory MSCs⁶⁰ — which comprise a heterogeneous population, including mature granulocytes, monocytes and varying numbers of immature cells of the myelomonocytic lineage (BOX 1) — are generated and recruited during the progression of chronic inflammation. The various cells secrete metabolites, such as nitric oxide (NO), hydrogen peroxide and prostaglandin E₂ (REFS 80,81), that negatively affect T-cell function. Several studies have shown that activated macrophages isolated from tumour-bearing hosts can induce downregulation of ζ -chain expression and impairment of T-cell function by secreting oxidants^{85,86}. Others have shown that prostaglandin E₂ — a product of arachidonic-acid metabolism, which is produced by macrophages at sites of inflammation or tissue damage — inhibits T-cell activation and proliferation^{87,88}. In addition, several studies indicate a central role for L-arginine catabolism in the suppressor mechanism of MSCs^{60,61,89}. L-Arginine is essential for T-cell proliferation and function. Its consumption by mature macrophages and MSCs (BOX 1) results in loss of ζ -chain expression, impaired T-cell proliferation and decreased cytokine production in response to antigen.

In the immunosuppressive environment, various pro-inflammatory cytokines are secreted that directly or indirectly affect T cells. For example, during an excessive T_H1 -cell immune response, the pro-inflammatory cytokine IFN- γ is continuously secreted. Studies carried out in my laboratory indicate that IFN- γ indirectly affects T cells by the recruitment and/or generation, and activation, of regulatory MSCs⁵⁹. In turn, the MSCs establish an inhibitory environment (FIG. 3), most probably by consuming L-arginine and by secreting NO and additional metabolites and cytokines, all of which contribute to the inhibitory surroundings that negatively affect T cells. In contrast to IFN- γ , which indirectly confers its inhibitory effect on T cells, TNF was proposed to directly affect T cells by inducing downregulation of ζ -chain expression. Consequently, the TCR-CD3 complex in these cells shows impaired assembly and stability at the cell surface, thereby uncoupling the TCR signal-transduction pathways^{52,78,90}. Further as-yet-unidentified factors could also be involved in suppressing T-cell responses.

The affected T cells. The mechanism by which the inhibitory environment directly affects ζ -chain expression is unknown. However, under the immunosuppressive conditions that are generated during infection, cancer and autoimmunity, various mechanisms for the induction of decreased ζ -chain expression have been proposed. My research group has shown that ζ -chain mRNA levels are not affected under chronic inflammatory conditions⁵⁹. Instead, a post-translational mechanism exists that specifically targets the ζ -chain for lysosomal degradation under these conditions. This mode of downregulation of ζ -chain expression was also observed in T cells that were isolated from tumour-bearing hosts⁹¹ and hosts with SLE⁹². Proteasome-dependent degradation was not detected in any of the cases that were analysed, except in a single study examining downregulation of ζ -chain expression in acute T-cell lymphoblastic leukaemia⁴⁰. It is not yet known why and how only the ζ -chain is targeted for lysosomal degradation and which molecules participate in this process. Phosphorylation-dependent ubiquitylation of transmembrane receptors leads to their internalization and, in many cases, to their lysosomal degradation. This mechanism attenuates receptor-mediated intracellular signalling^{93,94}. So, the tag for ζ -chain lysosomal degradation could result from ubiquitylation (either mono- or polyubiquitylation) that occurs after its unique phosphorylation under chronic inflammatory conditions. The pattern of ζ -chain phosphorylation during chronic inflammation is expected to differ from that observed in resting naive T cells (that is, the 21-kDa phosphorylated ζ -form), which has been suggested to confer survival signals²⁵. It has previously been shown that the ζ -chain undergoes activation-dependent ubiquitylation in normal T cells⁹⁵. However, the functional importance of this event has not yet been clarified. Alternatively, under inflammatory conditions, a novel protein could bind specifically to the modified or unmodified ζ -chain, or a protective

protein could dissociate from the ζ -chain. The ζ -chain could then be specifically targeted for degradation by any of these putative pathways.

Additional studies analysing peripheral-blood T cells from patients with gastric cancer indicate that increased caspase-3 activity in these cells might be responsible for the decreased levels of ζ -chain expression that occur together with increased T-cell apoptosis⁹⁶. It has also been shown that the ζ -chain has putative recognition sequences for caspase-3-related proteases⁹⁷. However, in most studies (including ours), T-cell apoptosis did not accompany downregulation of ζ -chain expression. By contrast, T-cell unresponsiveness was observed despite normal T-cell numbers. Further experiments are required to determine the link between oxidative stress, caspase-3 activation, ζ -chain expression downregulation and T-cell hyporesponsiveness.

By analysing patients with SLE, it was shown that downregulation of ζ -chain expression could result from changes at the level of transcription⁹⁸, as well as its lysosomal degradation⁹². In these cells, the level of activated ELF1 (E74-like factor 1) — the transcription factor that controls ζ -chain gene transcription — is reduced, thereby leading to decreased ζ -chain expression⁹⁸. It was also shown that ζ -chain mRNA that has an alternatively spliced 3'-untranslated region is the main form present in T cells of some patients with SLE, and this form shows reduced stability compared with wild-type ζ -chain mRNA⁹⁹. These studies indicate that in T cells isolated from patients with various pathologies, multiple mechanisms could induce the loss of ζ -chain expression. These mechanisms are not necessarily mutually exclusive, and they most probably occur because of differences in the physiology and duration of each pathology. However, in each of these cases, the resulting change is the reduction of ζ -chain expression. The mechanisms by which environmental factors induce the initiation of T-cell processes that are involved in the downregulation of ζ -chain expression are only just beginning to be studied.

Clinical implications

In many of the described pathologies, immunotherapeutic regimens are used to increase the immune response of the host and to induce disease regression. Some of these immunotherapies are based on peptide therapies⁶⁷ or vaccinations^{63,100–102}, and other strategies include T-cell-mediated immunotherapies^{100,103}. In all cases, the expectation is either that the immune system of the host will be activated (after vaccination) or that the administered T cells will function in the affected host (after T-cell therapy). In many cases, such immunotherapies administered to tumour-bearing patients or patients who suffer from chronic infection are not successful. The immunosuppressive environment generated in these patients during chronic inflammation seems not only to prevent the response to vaccination but also to downregulate ζ -chain expression in the newly administered T cells. Moreover, it is possible that adjuvants that are used to enhance the immune response of patients to weakly immunogenic

tumour- or pathogen-derived antigens might even result in activation of the regulatory MSCs. So, blockade of negative-regulatory pathways might be required to potentiate the effect of immunotherapies. Accordingly, the timing of therapies must also be reassessed. Such considerations could be based on measurements of ζ -chain expression by the peripheral T cells of the host: that is, ζ -chain expression could be used as a prognostic marker for the appearance of an immunosuppressive environment and to measure the impact of the disease on the immune system of patients¹⁰⁴. If downregulation of ζ -chain expression is detected, agents that neutralize the immunosuppressive factors and/or cells, or induce ζ -chain re-expression, need to be administered to patients before, or together with, the immunotherapy. However, treatment with neutralizing modalities must be selective to avoid inhibiting cells that are crucial for the induction of a functional immune response.

For example, neutralization of the immunosuppressive function of immature MSCs has been achieved both *in vitro* and *in vivo* by inducing their differentiation with granulocyte/macrophage colony-stimulating factor (GM-CSF)¹⁰⁵. However, cytokines that induce MSC differentiation *in vitro* might have limited use *in vivo* due to their pleiotropic activity and the secondary effects that they might exert in patients with chronic disease. For example, GM-CSF is one of the key cytokines responsible for myelopoiesis. So, if it is administered at an inappropriate dose, myeloid progenitors are renewed and the blockade of this population is not effective. Moreover, in conjunction with other cytokines, GM-CSF functions as a growth factor for erythroid and megakaryocyte progenitors. So, careful development of treatment protocols is required when using such a pleiotropic cytokine¹⁰⁶.

An alternative strategy with similar risks is the use of blocking antibodies specific for the cytokines and chemokines that are involved in the recruitment and/or activation of MSCs^{107,108} or are secreted by MSCs. However, because there are numerous cytokines that have a similar redundant activity on MSCs, this approach might require the combination of several cytokine-specific and possibly chemokine-specific antibodies. At present, treatment protocols that involve blocking antibodies specific for secreted cytokines are in use. For example, treatment of patients that have psoriasis with TNF-specific antibodies has resulted in long-lived remissions of the cutaneous manifestations¹⁰⁹. However, when these antibodies were administered together with cyclosporin, T-cell lymphomas were induced¹¹⁰. The treatment of patients that have rheumatoid arthritis with TNF-specific or IFN- γ -specific antibodies improved the quality of life of these patients^{111,112}. Therefore, the use of blocking antibodies such as these seems promising, but their administration in combination with immunosuppressive reagents warrants further investigation.

One promising therapeutic strategy would be to target the mechanisms (that is, the metabolites) that MSCs use to suppress T-cell function. For example, compounds that affect **arginase-1** and **inducible NO**

synthase activity might form a novel class of immune modulators that would function by limiting the effects of MSC activity *in vivo*^{113,114}. Therefore, further identification and characterization of both the subpopulations within MSCs and the factors that function in the immunosuppressive environment might enable the development of promising treatments to neutralize this environment that could be used in conjunction with immunotherapy. Identification of the T-cell molecules and/or signalling pathways that are involved in the specific induction of downregulation of ζ -chain expression could also lead to the development of modalities to block these pathways. Several studies indicate that treatment of affected T cells with interleukin-2 (IL-2) increases ζ -chain expression and restores T-cell function^{115,116}. However, these results are contentious, because in many other cases, IL-2 did not have the same effect^{117,118}. There are several possible reasons for this discrepancy, one of which is that IL-2 could affect T cells to different extents depending on the severity of the pathology and the state of the chronic inflammation. Another probable explanation is that in most cases in which IL-2 was observed to have an effect, the T cells were removed from the immunosuppressive environment and then tested *in vitro* in the presence of IL-2 (REFS 54,115). My research group has shown that IL-2 does not restore ζ -chain expression of affected T cells when they are co-cultured with immunosuppressive myeloid cells. However, T cells that are isolated from the immunosuppressive environment will eventually regain ζ -chain expression even when IL-2 is absent from the culture (M.B., unpublished observations). IL-2 might expedite the recovery *in vitro*, but its effect *in vivo* in the presence of a chronic inflammatory background must be more extensively studied.

Concluding remarks

Our knowledge of the phenomenon of downregulation of ζ -chain expression and the ensuing unresponsiveness of T cells provides new ways for understanding how the immune system functions on a background of acute and chronic inflammatory responses. The tight regulation of T-cell activation, and the integrated mechanisms that activate or attenuate T-cell function under such conditions, control the level and magnitude of the response. In most cases, inflammatory responses benefit the host by clearance of the pathogen, or the pathogen-infected or altered-self cells. If acute inflammation develops, it is accompanied by a transient attenuation of T-cell function that is associated with downregulation of TCR ζ -chain expression. Most episodes of inflammation resolve spontaneously, and the T cells regain ζ -chain expression and responsiveness. However, in many diseases — such as cancer, and autoimmune and infectious diseases — the inflammatory response persists, and a stable infiltration of inflammatory cells accumulates in the tissues and lymphatic organs, leading to a chronic immunosuppressive environment that continuously attenuates T-cell function. In this situation, host T cells lose ζ -chain expression and become non-functional. So, chronic inflammation

generates a potentially vicious self-sustaining loop(s), which results in an immunocompromised microenvironment that is supportive of cancer, chronic infection and damage to self tissue. So far, much knowledge has been gained to support the hypothesis that the observed downregulation of ζ -chain expression and impairment of T-cell function are normal responses of the immune system, which have evolved to attenuate T-cell function and overcome the exacerbated activation that occurs in a developing chronic inflammatory environment. Despite the immunosuppressive activity of some tumour-derived factors, I think that downregulation of ζ -chain expression and impairment of T-cell function are not the consequence of an escape mechanism that tumours and pathogens have evolved. Instead, downregulation of ζ -chain expression is a natural outcome of the homeostatic regulation of the immune response. This phenomenon has a major impact on current immunotherapies, because an immunosuppressive environment might obstruct the efficacy of vaccinations and T-cell therapies.

With our greater understanding of this suppressive mechanism, efforts to optimize immunotherapy must be directed along two routes. On the clinical front, it is already possible to evaluate the immunosuppressive environment in patients before treatment with a particular immunotherapy. However, further studies are required to identify and more-precisely characterize the immunosuppressive cells and factors. These could enable the future development of modalities to prevent suppression of ζ -chain expression during the course of a given treatment. On the preclinical front, studies need to focus on the molecules that are activated in T cells under chronic inflammatory conditions and on the mechanisms by which the immunosuppressive cells and factors affect T cells and induce downregulation of ζ -chain expression. Together, these approaches promise to advance our understanding of the mechanisms by which chronic inflammatory immune responses contribute to various pathologies and how these responses can be artificially regulated and controlled.

1. Germain, R. N. & Stefanova, I. The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation. *Annu. Rev. Immunol.* **17**, 467–522 (1999).
2. Samelson, L. E. Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. *Annu. Rev. Immunol.* **20**, 371–394 (2002).
3. Koretzky, G. A. & Myung, P. S. Positive and negative regulation of T-cell activation by adaptor proteins. *Nature Rev. Immunol.* **1**, 95–107 (2001).
4. Leo, A., Wienands, J., Baier, G., Horejsi, V. & Schraven, B. Adapters in lymphocyte signaling. *J. Clin. Invest.* **109**, 301–309 (2002).
5. Alarcon, B., Gil, D., Delgado, P. & Schamel, W. W. Initiation of TCR signaling: regulation within CD3 dimers. *Immunol. Rev.* **191**, 38–46 (2003).
6. Dustin, M. L. Coordination of T cell activation and migration through formation of the immunological synapse. *Ann. NY Acad. Sci.* **987**, 51–59 (2003).
7. Huppa, J. B. & Davis, M. M. T-cell-antigen recognition and the immunological synapse. *Nature Rev. Immunol.* **3**, 973–983 (2003).
8. Janeway, C. A., Travers, P., Walport, M. & Shlomchik, M. *Immunobiology* 1–423 (Garland, New York, 2001).
9. Valitutti, S., Muller, S., Sallio, M. & Lanzavecchia, A. Degradation of T cell receptor (TCR)-CD3- ζ complexes after antigenic stimulation. *J. Exp. Med.* **185**, 1859–1864 (1997).
10. D'Oro, U., Vacchio, M. S., Weissman, A. M. & Ashwell, J. D. Activation of the Lck tyrosine kinase targets cell surface T cell antigen receptors for lysosomal degradation. *Immunity* **7**, 619–628 (1997).
11. Bronstein-Sitton, N., Wang, L., Cohen, L. & Baniyash, M. Expression of the T cell antigen receptor ζ chain following activation is controlled at distinct checkpoints. Implications for cell surface receptor down-modulation and re-expression. *J. Biol. Chem.* **274**, 23659–23665 (1999).
12. Viola, A. & Lanzavecchia, A. T cell activation determined by T cell receptor number and tunable thresholds. *Science* **273**, 104–106 (1996).
13. Chikuma, S. & Bluestone, J. A. CTLA-4 and tolerance: the biochemical point of view. *Immunol. Res.* **28**, 241–253 (2003).
14. Jang, I. K. & Gu, H. Negative regulation of TCR signaling and T-cell activation by selective protein degradation. *Curr. Opin. Immunol.* **15**, 315–320 (2003).
15. Samelson, L. E., Harford, J. B. & Klausner, R. D. Identification of the components of the murine T cell antigen receptor complex. *Cel* **43**, 223–231 (1985).
16. Weissman, A. M. *et al.* Molecular cloning of the ζ chain of the T cell antigen receptor. *Science* **239**, 1018–1021 (1988).
17. Lanier, L. L., Yu, G. & Phillips, J. H. Co-association of CD3 ζ with a receptor (CD16) for IgG Fc on human natural killer cells. *Nature* **342**, 803–805 (1989).
18. Moretta, A. *et al.* Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu. Rev. Immunol.* **19**, 197–223 (2001).
19. Baniyash, M., Hsu, V. W., Seldin, M. F. & Klausner, R. D. The isolation and characterization of the murine T cell antigen receptor ζ chain gene. *J. Biol. Chem.* **264**, 13252–13257 (1989).
20. Pitcher, L. A. *et al.* The formation and functions of the 21- and 23-kDa tyrosine-phosphorylated TCR ζ subunits. *Immunol. Rev.* **191**, 47–61 (2003).
21. Caplan, S., Zelig, S., Wang, L. & Baniyash, M. Cell-surface-expressed T-cell antigen-receptor ζ chain is associated with the cytoskeleton. *Proc. Natl Acad. Sci. USA* **92**, 4768–4772 (1995).
22. Rozdzial, M. M., Malissen, B. & Finkel, T. H. Tyrosine-phosphorylated T cell receptor ζ chain associates with the actin cytoskeleton upon activation of mature T lymphocytes. *Immunity* **3**, 623–633 (1995).
23. D'Oro, U. *et al.* Regulation of constitutive TCR internalization by the ζ -chain. *J. Immunol.* **169**, 6269–6278 (2002).
24. Klausner, R. D., Lippincott-Schwartz, J. & Bonifacio, J. S. The T cell antigen receptor: insights into organelle biology. *Annu. Rev. Cell Biol.* **6**, 403–431 (1990).
25. Witherden, D. *et al.* Tetracycline-controllable selection of CD4⁺ T cells: half-life and survival signals in the absence of major histocompatibility complex class II molecules. *J. Exp. Med.* **191**, 355–364 (2000).
26. Mizoguchi, H. *et al.* Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* **258**, 1795–1798 (1992).
27. Matsuda, M. *et al.* Alterations in the signal-transducing molecules of T cells and NK cells in colorectal tumor-infiltrating, gut mucosal and peripheral lymphocytes: correlation with the stage of the disease. *Int. J. Cancer* **61**, 765–772 (1995).
28. Kiessling, R. Signals from lymphocytes in colon cancer. *Gut* **40**, 153–154 (1997).
29. Finke, J. H. *et al.* Loss of T-cell receptor ζ chain and p56^{lck} in T-cells infiltrating human renal carcinoma. *Cancer Res.* **53**, 5613–5616 (1993).
30. Bukowski, R. M. *et al.* Signal transduction abnormalities in T lymphocytes from patients with advanced renal carcinoma: clinical relevance and effects of cytokine therapy. *Clin. Cancer Res.* **4**, 2337–2347 (1998).
31. Kuss, I., Rabinowich, H., Gooding, W., Edwards, R. & Whiteside, T. L. Expression of ζ in T cells prior to interleukin-2 therapy as a predictor of response and survival in patients with ovarian carcinoma. *Cancer Biother. Radiopharm.* **17**, 631–640 (2002).
32. Lai, P. *et al.* Alterations in expression and function of signal-transducing proteins in tumor-associated T and natural killer cells in patients with ovarian carcinoma. *Clin. Cancer Res.* **2**, 161–173 (1996).
33. Kono, K. *et al.* Decreased expression of signal-transducing ζ chain in peripheral T cells and natural killer cells in patients with cervical cancer. *Clin. Cancer Res.* **2**, 1825–1828 (1996).
34. Kurt, R. A., Urba, W. J., Smith, J. W. & Schoof, D. D. Peripheral T lymphocytes from women with breast cancer exhibit abnormal protein expression of several signaling molecules. *Int. J. Cancer* **78**, 16–20 (1998).
35. Schule, J., Bergkvist, L., Hakansson, L., Gustafsson, B. & Hakansson, A. Down-regulation of the CD3- ζ chain in sentinel node biopsies from breast cancer patients. *Breast Cancer Res. Treat.* **74**, 33–40 (2002).
36. Healy, C. G. *et al.* Impaired expression and function of signal-transducing ζ chains in peripheral T cells and natural killer cells in patients with prostate cancer. *Cytometry* **32**, 109–119 (1998).
37. Kuss, I., Saito, T., Johnson, J. T. & Whiteside, T. L. Clinical significance of decreased ζ chain expression in peripheral blood lymphocytes of patients with head and neck cancer. *Clin. Cancer Res.* **5**, 329–334 (1999).
38. Zea, A. H. *et al.* Alterations in T cell receptor and signal transduction molecules in melanoma patients. *Clin. Cancer Res.* **1**, 1327–1335 (1995).
39. Dworacki, G. *et al.* Decreased ζ chain expression and apoptosis in CD3⁺ peripheral blood T lymphocytes of patients with melanoma. *Clin. Cancer Res.* **7**, 947S–957S (2001).
40. Torelli, G. F. *et al.* Defective expression of the T-cell receptor-CD3 ζ chain in T-cell acute lymphoblastic leukaemia. *Br. J. Haematol.* **120**, 201–208 (2003).
41. Chen, X. *et al.* Impaired expression of the CD3- ζ chain in peripheral blood T cells of patients with chronic myeloid leukaemia results in an increased susceptibility to apoptosis. *Br. J. Haematol.* **111**, 817–825 (2000).
42. Massaia, M., Attisano, C., Beggato, E., Bianchi, A. & Pileri, A. Correlation between disease activity and T-cell CD3 ζ chain expression in a B-cell lymphoma. *Br. J. Haematol.* **88**, 886–888 (1994).
43. Frydecka, I. *et al.* Expression of signal-transducing ζ chain in peripheral blood T cells and natural killer cells in patients with Hodgkin's disease in different phases of the disease. *Leuk. Lymphoma* **35**, 545–554 (1999).
44. Schmielau, J., Nalesnik, M. A. & Finn, O. J. Suppressed T-cell receptor ζ chain expression and cytokine production in pancreatic cancer patients. *Clin. Cancer Res.* **7**, 933S–939S (2001).
45. Ungefroren, H. *et al.* Immunological escape mechanisms in pancreatic carcinoma. *Ann. NY Acad. Sci.* **880**, 243–251 (1999).
46. Hirst, W., Buggins, A. & Mufti, G. Central role of leukemia-derived factors in the development of leukemia-associated immune dysfunction. *Hematol. J.* **2**, 2–17 (2001).
47. Taylor, D. D., Bender, D. P., Gerceci-Taylor, C., Stanson, J. & Whiteside, T. L. Modulation of TCR/CD3- ζ chain expression by a circulating factor derived from ovarian cancer patients. *Br. J. Cancer* **84**, 1624–1629 (2001).

48. Lioussis, S. N., Ding, X. Z., Dennis, G. J. & Tsokos, G. C. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor ζ chain. *J. Clin. Invest.* **101**, 1448–1457 (1998).
49. Tsokos, G. C., Wong, H. K., Enyedy, E. J. & Nambiar, M. P. Immune cell signaling in lupus. *Curr. Opin. Rheumatol.* **12**, 355–363 (2000).
50. Maurice, M. M. *et al.* Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis. *J. Immunol.* **159**, 2973–2978 (1997).
51. Romagnoli, P., Strahan, D., Pelosi, M., Cantagrel, A. & van Meerwijk, J. P. A potential role for protein tyrosine kinase p56^{lck} in rheumatoid arthritis synovial fluid T lymphocyte hyporesponsiveness. *Int. Immunol.* **13**, 305–312 (2001).
52. Cope, A. P. Studies of T-cell activation in chronic inflammation. *Arthritis Res.* **4**, S197–S211 (2002).
53. Stefanova, I. *et al.* HIV infection-induced posttranslational modification of T cell signaling molecules associated with disease progression. *J. Clin. Invest.* **98**, 1290–1297 (1996).
54. Trimble, L. A. & Lieberman, J. Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3 ζ , the signaling chain of the T-cell receptor complex. *Blood* **91**, 585–594 (1998).
55. Geertsma, M. F., Stevenhagen, A., van Dam, E. M. & Nibbering, P. H. Expression of ζ molecules is decreased in NK cells from HIV-infected patients. *FEMS Immunol. Med. Microbiol.* **26**, 249–257 (1999).
56. Zea, A. H. *et al.* Changes in expression of signal transduction proteins in T lymphocytes of patients with leprosy. *Infect. Immun.* **66**, 499–504 (1998).
57. Sakaguchi, N. *et al.* Altered thymic T-cell selection due to a mutation of the *Zap-70* gene causes autoimmune arthritis in mice. *Nature* **426**, 454–460 (2003).
58. Tsokos, G. C., Nambiar, M. P., Tenbroeck, K. & Juang, Y. T. Rewiring the T-cell: signaling defects and novel prospects for the treatment of SLE. *Trends Immunol.* **24**, 259–263 (2003).
59. Bronstein-Sitton, N. *et al.* Sustained exposure to bacterial antigen induces interferon- γ -dependent T cell receptor ζ down-regulation and impaired T cell function. *Nature Immunol.* **4**, 957–964 (2003).
- This paper describes a model system that mimics the inflammatory conditions generated in various pathologies that lead to downregulation of ζ -chain expression and hyporesponsiveness of T cells. These conditions are achieved by sustained exposure of mice to an antigen that induces an IFN- γ -dependent T_H1-cell immune response.**
60. Serafini, P. *et al.* Derangement of immune responses by myeloid suppressor cells. *Cancer Immunol. Immunother.* **53**, 64–72 (2004).
- This review examines recent findings about the nature, properties and mechanisms of action of MSCs, which have an important role in the creation of an immunosuppressive environment.**
61. Goni, O., Alcaide, P. & Fresno, M. Immunosuppression during acute *Trypanosoma cruzi* infection: involvement of Ly6G⁺(Gr1⁺)CD11b⁺ immature myeloid suppressor cells. *Int. Immunol.* **14**, 1125–1134 (2002).
62. Gretzer, M. B. & Partin, A. W. PSA markers in prostate cancer detection. *Urol. Clin. North Am.* **30**, 677–686 (2003).
63. Lollini, P. L. & Forni, G. Antitumor vaccines: is it possible to prevent a tumor? *Cancer Immunol. Immunother.* **51**, 409–416 (2002).
64. Taylor-Papadimitriou, J. *et al.* MUC1 and the immunobiology of cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 209–221 (2002).
65. Lee, S. P. Nasopharyngeal carcinoma and the EBV-specific T cell response: prospects for immunotherapy. *Semin. Cancer Biol.* **12**, 463–471 (2002).
66. Corrigan, V. M. & Panayi, G. S. Autoantigens and immune pathways in rheumatoid arthritis. *Crit. Rev. Immunol.* **22**, 281–293 (2002).
67. Monneaux, F. & Muller, S. Peptide-based immunotherapy of systemic lupus erythematosus. *Autoimmun. Rev.* **3**, 16–24 (2004).
68. Illei, G. G. & Czizjak, L. Novel approaches in the treatment of lupus nephritis. *Expert Opin. Invest. Drugs* **10**, 1117–1130 (2001).
69. Dabbagh, K. & Lewis, D. B. Toll-like receptors and T-helper-1/T-helper-2 responses. *Curr. Opin. Infect. Dis.* **16**, 199–204 (2003).
70. Johnson, G. B., Brunn, G. J., Tang, A. H. & Platt, J. L. Evolutionary clues to the functions of the Toll-like family as surveillance receptors. *Trends Immunol.* **24**, 19–24 (2003).
71. MacArthur, M., Hold, G. L. & El-Omar, E. M. Inflammation and cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am. J. Physiol. Gastrointest. Liver Physiol.* **286**, G515–G520 (2004).
72. Ness, R. B. & Cottréau, C. Possible role of ovarian epithelial inflammation in ovarian cancer. *J. Natl Cancer Inst.* **91**, 1459–1467 (1999).
73. Castle, P. E. & Giuliano, A. E. Genital tract infections, cervical inflammation, and antioxidant nutrients — assessing their roles as human papillomavirus cofactors. *J. Natl Cancer Inst. Monogr.* **31**, 29–34 (2003).
74. Lin, E. Y. & Pollard, J. W. Macrophages: modulators of breast cancer progression. *Novartis Found. Symp.* **256**, 158–168 (2004).
75. Platz, E. A. & De Marzo, A. M. Epidemiology of inflammation and prostate cancer. *J. Urol.* **171**, S36–S40 (2004).
76. Maisonneuve, P. & Lowenfels, A. B. Chronic pancreatitis and pancreatic cancer. *Dig. Dis.* **20**, 32–37 (2002).
77. Tsokos, G. C. & Lioussis, S. N. Lymphocytes, cytokines, inflammation, and immune trafficking. *Curr. Opin. Rheumatol.* **10**, 417–425 (1998).
78. Cope, A. P. Exploring the reciprocal relationship between immunity and inflammation in chronic inflammatory arthritis. *Rheumatology* **42**, 716–731 (2003).
79. Buckley, C. D. Why does chronic inflammatory joint disease persist? *Clin. Med.* **3**, 361–366 (2003).
80. Ohshima, H., Tatemichi, M. & Sawa, T. Chemical basis of inflammation-induced carcinogenesis. *Arch. Biochem. Biophys.* **417**, 3–11 (2003).
81. Schwartsburd, P. M. Chronic inflammation as inductor of pro-cancer microenvironment: pathogenesis of dysregulated feedback control. *Cancer Metastasis Rev.* **22**, 95–102 (2003).
82. Thun, M. J., Henley, S. J. & Gansler, T. Inflammation and cancer: an epidemiological perspective. *Novartis Found. Symp.* **256**, 6–21 (2004).
- References 80–82 highlight the link between chronic inflammatory conditions and increased risk of cancer in affected tissues. During chronic inflammation, complex microenvironmental changes are induced, which are similar to those observed near growing cancer cells. These changes result in the inhibition of immune cells and the generation of a microenvironment that favours the growth and survival of tumour cells.**
83. Mühlestein, J. B. & Anderson, J. L. Chronic infection and coronary artery disease. *Cardiol. Clin.* **21**, 333–362 (2003).
84. Nambiar, M. P., Mitchell, J. P., Ceruti, R. P., Malloy, M. A. & Tsokos, G. C. Prevalence of T cell receptor ζ chain deficiency in systemic lupus erythematosus. *Lupus* **12**, 46–51 (2003).
85. Kono, K. *et al.* Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing ζ molecules and inhibits tumor-specific T cell- and natural killer cell-mediated cytotoxicity. *Eur. J. Immunol.* **26**, 1308–1313 (1996).
86. Otsuji, M., Kimura, Y., Aoe, T., Okamoto, Y. & Saito, T. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 ζ chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc. Natl Acad. Sci. USA* **93**, 13119–13124 (1996).
87. Naama, H. A., Mack, V. E., Smyth, G. P., Stapleton, P. P. & Daly, J. M. Macrophage effector mechanisms in melanoma in an experimental study. *Arch. Surg.* **136**, 804–809 (2001).
88. Yaqub, S. *et al.* A human whole blood model of LPS-mediated suppression of T cell activation. *Med. Sci. Monit.* **9**, BR120–BR126 (2003).
89. Rodriguez, P. C. *et al.* L-Arginine consumption by macrophages modulates the expression of CD3 ζ chain in T lymphocytes. *J. Immunol.* **171**, 1232–1239 (2003).
- This study shows that macrophages stimulated with IL-4 and IL-13 upregulate arginase-1 and cationic amino-acid transporter 2B, thereby causing a rapid reduction in the extracellular levels of L-arginine, decreased expression of the ζ -chain and diminished proliferation of normal T cells.**
90. Isomaki, P. *et al.* Prolonged exposure of T cells to TNF down-regulates TCR ζ and expression of the TCR/CD3 complex at the cell surface. *J. Immunol.* **166**, 5495–5507 (2001).
91. Correa, M. R. *et al.* Sequential development of structural and functional alterations in T cells from tumor-bearing mice. *J. Immunol.* **158**, 5292–5296 (1997).
92. Brundula, V. *et al.* Diminished levels of T cell receptor ζ chains in peripheral blood T lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 1908–1916 (1999).
93. Hicke, L. & Dunn, R. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu. Rev. Cell Dev. Biol.* **19**, 141–172 (2003).
94. Haglund, K., Di Fiore, P. P. & Dikic, I. Distinct monoubiquitin signals in receptor endocytosis. *Trends Biochem. Sci.* **28**, 598–603 (2003).
- This review summarizes new concepts about protein ubiquitylation, indicating that the type of ubiquitylation dictates the functional consequences. Whereas polyubiquitylated proteins are targeted for degradation by the proteasome, the attachment of several monoubiquitin molecules to a protein might provide a targeting signal that ensures efficient endocytic sorting and lysosomal degradation.**
95. Hou, D., Cenciarelli, C., Jensen, J. P., Nguyen, H. B. & Weissman, A. M. Activation-dependent ubiquitination of a T cell antigen receptor subunit on multiple intracellular lysines. *J. Biol. Chem.* **269**, 14244–14247 (1994).
96. Takahashi, A. *et al.* Elevated caspase-3 activity in peripheral blood T cells coexists with increased degree of T-cell apoptosis and down-regulation of TCR ζ molecules in patients with gastric cancer. *Clin. Cancer Res.* **7**, 74–80 (2001).
97. Gastman, B. R., Johnson, D. E., Whiteside, T. L. & Rabinowich, H. Caspase-mediated degradation of T-cell receptor ζ -chain. *Cancer Res.* **59**, 1422–1427 (1999).
98. Tsokos, G. C., Nambiar, M. P. & Juang, Y. T. Activation of the Ets transcription factor Elf-1 requires phosphorylation and glycosylation: defective expression of activated Elf-1 is involved in the decreased TCR ζ chain gene expression in patients with systemic lupus erythematosus. *Ann. NY Acad. Sci.* **987**, 240–245 (2003).
99. Tszuzaka, K. *et al.* TCR ζ mRNA with an alternatively spliced 3'-untranslated region detected in systemic lupus erythematosus patients leads to the down-regulation of TCR ζ and TCR/CD3 complex. *J. Immunol.* **171**, 2496–2503 (2003).
- References 98 and 99 indicate the different mechanisms that are involved in the induction of downregulation of ζ -chain expression in T cells isolated from patients with SLE. Reference 98 reports a transcriptional control mechanism. Reference 99 reports a post-transcriptional control mechanism that involves the generation of an alternatively spliced ζ -chain mRNA 3'-untranslated region, which leads to instability. An additional, post-translational, control mechanism that involves enhanced lysosomal degradation of the ζ -chain is reported in reference 92.**
100. Rosenberg, S. A. Shedding light on immunotherapy for cancer. *N. Engl. J. Med.* **350**, 1461–1463 (2004).
101. Kadison, A. S. & Morton, D. L. Immunotherapy of malignant melanoma. *Surg. Clin. North Am.* **83**, 343–370 (2003).
102. Horig, H., Lee, C. S. & Kaufman, H. L. Prostate-specific antigen vaccines for prostate cancer. *Expert Opin. Biol. Ther.* **2**, 395–408 (2002).
103. Abken, H., Hombach, A. & Heuser, C. Immune response manipulation: recombinant immunoreceptors endow T cells with predefined specificity. *Curr. Pharm. Des.* **9**, 1992–2001 (2003).
104. Whiteside, T. L. Down-regulation of ζ -chain expression in T cells: a biomarker of prognosis in cancer? *Cancer Immunol. Immunother.* **29**, 29–34 (2004).
- This review highlights the putative clinical relevance of ζ -chain expression as an immunological biomarker of favourable responses to biological therapies, indicating that ζ -chain expression levels could help in the selection of patients for immunotherapy trials.**
105. Li, Q., Pan, P. Y., Gu, P., Xu, D. & Chen, S. H. Role of immature myeloid Gr-1⁺ cells in the development of antitumor immunity. *Cancer Res.* **64**, 1130–1139 (2004).
- This paper indicates that GM-CSF treatment that induces maturation of immature myeloid cells prevents the inhibitory activity of these cells and enhances their antigen-presenting capability. Therefore, gene therapy using GM-CSF could induce effective antitumor responses in hosts with advanced tumours.**
106. Barreda, D. R., Hanington, P. C. & Belosevic, M. Regulation of myeloid development and function by colony stimulating factors. *Dev. Comp. Immunol.* **28**, 509–554 (2004).
107. Ahlers, J. D. *et al.* A push–pull approach to maximize vaccine efficacy: abrogating suppression with an IL-13 inhibitor while augmenting help with granulocyte/macrophage colony-stimulating factor and CD40L. *Proc. Natl Acad. Sci. USA* **99**, 13020–13025 (2002).
108. Almand, B. *et al.* Clinical significance of defective dendritic cell differentiation in cancer. *Clin. Cancer Res.* **6**, 1755–1766 (2000).
109. Gottlieb, A. B. Clinical research helps elucidate the role of tumor necrosis factor- α in the pathogenesis of T1-mediated immune disorders: use of targeted immunotherapeutics as pathogenic probes. *Lupus* **12**, 190–194 (2003).

110. Mahe, E., Descamps, V., Grossin, M., Fraïtag, S. & Crickx, B. CD30⁺ T-cell lymphoma in a patient with psoriasis treated with ciclosporin and infliximab. *Br. J. Dermatol.* **149**, 170–173 (2003).
111. Fong, K. Y. Immunotherapy in autoimmune diseases. *Ann. Acad. Med. Singapore* **31**, 702–706 (2002).
112. Sigidin, Y. A., Loukina, G. V., Skurkovich, B. & Skurkovich, S. Randomized, double-blind trial of anti-interferon- γ antibodies in rheumatoid arthritis. *Scand. J. Rheumatol.* **30**, 203–207 (2001).
113. Colleluori, D. M. & Ash, D. E. Classical and slow-binding inhibitors of human type II arginase. *Biochemistry* **40**, 9356–9362 (2001).
114. Vallance, P. & Leiper, J. Blocking NO synthesis: how, where and why? *Nature Rev. Drug Discov.* **1**, 939–950 (2002).
115. Seitzer, U. *et al.* Reduced T-cell receptor CD3 ζ -chain protein and sustained CD3 ϵ expression at the site of mycobacterial infection. *Immunology* **104**, 269–277 (2001).
116. Salvadori, S., Gansbacher, B., Pizzimenti, A. M. & Zier, K. S. Abnormal signal transduction by T cells of mice with parental tumors is not seen in mice bearing IL-2-secreting tumors. *J. Immunol.* **153**, 5176–5182 (1994).
117. Farace, F., Angevin, E., Vanderplancke, J., Escudier, B. & Triebel, F. The decreased expression of CD3 ζ chains in cancer patients is not reversed by IL-2 administration. *Int. J. Cancer* **59**, 752–755 (1994).
118. Trimble, L. A., Shankar, P., Patterson, M., Daily, J. P. & Lieberman, J. Human immunodeficiency virus-specific circulating CD8 T lymphocytes have down-modulated CD3 ζ and CD28, key signaling molecules for T-cell activation. *J. Virol.* **74**, 7320–7330 (2000).
119. Mazzone, A. *et al.* Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. *J. Immunol.* **168**, 689–695 (2002).
120. Wu, G. & Morris, S. M. Jr. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* **336**, 1–17 (1998).
121. Jaffe, M. L., Arai, H. & Nabel, G. J. Mechanisms of tumor-induced immunosuppression: evidence for contact-dependent T cell suppression by monocytes. *Mol. Med.* **2**, 692–701 (1996).

Acknowledgements

I gratefully acknowledge the support of the Society of Research Associates of the Lautenberg Center, New York, United States, the Concern Foundation of Los Angeles, United States, and the Harold B. Abramson Chair in Immunology, Jerusalem, Israel. This study was supported by The Israel Academy of Sciences and Humanities, and by The Joseph and Matilda Melnick fund Houston, United States.

I thank S. Caplan, S. Schwarzbaum, L. Cohen-Daniel, E. Manaster and I. Vaknin for careful reading of the manuscript and constructive suggestions.

Competing interests statement

The author declares no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
arginase-1 | caspase-3 | CD3 γ | CD3 δ | CD3 ϵ | CD16 | Fc ϵ R γ | FYN | GM-CSF | GR1 | granzyme B | IFN- γ | inducible NO synthase | LCK | NKp30 | NKp46 | TNF | ZAP70 | ζ -chain

Infectious Disease Information:

<http://www.cdc.gov/ncidod/diseases/index.htm>
HIV | leprosy

OMIM:

<http://www.ncbi.nlm.nih.gov/Omim/>
rheumatoid arthritis | SLE

Access to this interactive links box is free online.