Transpl Int (1998) 11: 169–180

REVIEW ARTICLE

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Intragraft cytokine gene expression: implications for clinical transplantation

Received: 15 July 1997
Received after revision: 5 January 1998
Accepted: 14 January 1998

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Abstract As our knowledge of the cytokine network in experimental transplant models grows, we need to understand how and to what extent cytokines mediate the various donor-directed immune events in clinical situations. This overview of clinical cytokine measurements shows that specific intragraft cytokine messenger RNA (mRNA) expression profiles can be associated with acute rejection, that they may reflect the efficacy of immunosuppression, and that they can identify patients at risk for the development of early chronic rejection. The literature also shows that acute rejection and immunological quiescence in humans are not restricted to the cytokine patterns defined in the type 1/type 2 paradigm. This apparent lack of association may be caused by the immunosuppression used in the clinic but may also be the result of the infinite diversity of donor and recipient factors, in which polymorphisms in cytokines and cytokine receptor genes may play a central role.

Key words Intragraft cytokine mRNA, expression · Expression, intragraft cytokine mRNA

Introduction

Studies in animal transplant models have clearly proven the value of cytokine measurements. These studies have taught us a great deal about the cytokine network during the full spectrum of transplant-associated immune responses. However, it is important to understand to what extent these findings can be applied in the clinic and whether alterations in the cytokine pattern may help the clinician to adapt the immunosuppressive regimen in a given individual.

Measurement of cytokines became very popular with the introduction of the reverse transcriptase-polymerase chain reaction (RT-PCR) and the availability of enzyme-linked immunosorbent assay (ELISA) kits in the early 1990s. These techniques made it possible to monitor both cytokine messenger RNA (mRNA) expression in tissues and cytokine protein concentrations in body fluids. In situ hybridization, bioassays, and immunohistochemistry were also applied to determine the presence of cytokines. Research on all of these methods resulted in a tremendous number of publications on cytokine measurements at various stages of the immune response after transplantation. Indeed, studies in experimental animal transplant settings have shown that cytokines play key roles at each of these stages.

In contrast to the clear-cut data derived from studies conducted under strict, controlled laboratory conditions, the results in clinical transplant settings have proven to be much more difficult to interpret. It is already known from large database studies that graft rejection and patient survival are influenced by a wide variety of donor and recipient factors including age, accompanying diseases of both organ donor and recipient, prior blood transfusions, HLA compatibility match grade, and history of viral infections. Moreover, the variety of immunosuppressive regimens currently used in clinical transplantation can affect various steps in the cascade of immune activation, albeit not always to the same degree in each patient. Consequently, the variability between individual patients will affect the results of

cytokine measurements. Most of the data relating local cytokine production to immune mechanisms after clinical transplantation are based on in situ detection of transcriptional factors by RT-PCR. This technique is suited to measure a broad panel of cytokine mRNAs in small amounts of tissue, as in biopsies. These cytokine measurements may provide information on immune events (acute and chronic rejection and graft acceptance) in individual patients. This review summarizes these intragraft cytokine measurements after heart, liver, and kidney transplantation and discusses the typical pitfalls associated with patient-directed research that may influence the outcome of intragraft cytokine mRNA measurements.

Characteristics of cytokines

Cytokines are soluble polypeptides that function as paracrine or autocrine mediators acting over short distances and that regulate a variety of immune and inflammatory responses. Since the discovery of the T-cell growth factor, presently known as IL-2, in 1978, soluble mediators have become the subject of considerable research [30]. A complete network of interleukins, interferons, chemokines, and growth factors has been characterized. Cytokines secreted under normal, as well as pathological, conditions affect proliferation, differentiation, and the functioning of cells involved in numerous physiological processes. Their biological activity is mediated by specific membrane receptors that can be expressed on a variety of cell types. Cytokines can have stimulatory and inhibitory properties, and they may synergize or antagonize the action of individual components in the network. One factor may replace or compensate for the lack of another cytokine (redundancy), but in the context of a particular immune function, e.g., graft rejection individual cytokines may have a dominant role. In the field of organ transplantation, this may be especially true of T cytokines. In 1991, Romagnani showed that human T helper (Th) cells like mouse CD4 + T cells express functionally distinct cytokine profiles, and the Th1/Th2 paradigm was born [58, 73]. According to this theory, Th1 cells produce interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β and favor cellular immune responses, delayed type hypersensitivity, and macrophage activation while Th2 cells secrete IL-4, IL-5, IL-6, and IL-10, favor tolerance, and stimulate B-cell differentiation and antibody responses [63]. Recently, it has become evident that this dichotomy is not confined to CD4 helper T cells alone [57]. CD8 effector cells and δ T cells may also secrete cytokines in a polarized fashion. This has led to the more generalized nomenclature "type 1 and type 2 cytokines" [18].

Cytokine measurements in experimental transplant settings

Studies in experimental transplant settings have shown that immune responses to an organ are regulated by cytokine interactions [24, 36]. From these and other studies, we have learned that endothelium damage by ischemia, reperfusion, and surgery triggers nonspecific, inflammatory responses mediated by members of the different cytokine families [24, 31, 36, 38, 51]. Damaged endothelial cells release increased amounts of IL-1 β and IL-6, IFN-γ, the chemokine macrophage chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and MIP-1 β, IL-8, colony-stimulating factors, and multiple growth factors such as TNF-α, platelet-derived growth factors (PDGF), insulin growth factor-1, transforming growth factor (TGF)-α, and basic fibroblast growth factor (FGF) [4, 25, 70, 76]. Thus, as a result of the surgical procedure, a complete network of cytokines is already activated, even before allogeneic reactions can be encountered. Cytokine production by activated endothelium results in upregulated HLA expression and increased adherence of monocytes and T cells, which is followed by infiltration into surrounding tissue. For example, TNF-α and IL-1 β induce vascular endothelial cells to transcribe the vascular adhesion molecule-1 [50]. The triggering step is, therefore, crucial since nonspecific endothelium injury can be the factor initiating the development of acute and/or chronic graft rejection.

The first experimental studies that analyzed molecular pathways involved in acute rejection and graft acceptance showed that these mechanisms are dominated by intragraft production of either type 1 cytokines or type 2 cytokines, respectively. In rodents and cynomolgus monkeys, acute rejection was accompanied by intragraft type 1 (IL-2, IFN-γ) mRNA expression, suggesting that these cytokines control the rejection process after kidney, heart, and liver transplantation [24, 56, 89, 101]. Studies in experimental models also showed that graft acceptance can be identified on the basis of a clear intragraft mRNA profile. In the majority of these studies, graft acceptance was associated with diminished type 1 cytokine and enhanced type 2 cytokine production [16, 19, 77, 88]. However, it is evident from studies using genetically manipulated animals that type 1 cytokines are not always required for rejection and that type 2 cytokines do not always initiate permanent engraftment. IL-2 and IFN-γ knockout mice reject their transplants in the presence of type 2 cytokines [45, 85] and IL-4 knockout mice accept their grafts in the presence of type 1 cytokines [59]. These studies indicate that immunological phenomena such as graft rejection and acceptance are not exclusively restricted to the type1/type 2 dichotomy. Alternative and/or redundant pathways may contribute to the alloimmune response associated

with these phenomena. Interestingly, results from the double IL-2 and IL-4 knockout model suggest that T-cell growth factors produced by non-T cells are also able to induce allograft rejection [74].

Evidence of a significant role of cytokines in the etiology of chronic allograft rejection or transplant arteriosclerosis has been found in experimental transplant studies as well. These studies have focused on cytokine production by injured endothelial cells and smooth muscle cells and on chemokine production by activated macrophages. Semiquantitative RT-PCR analysis in rat aortic allografts has shown that mRNA expression of various cytokines (e.g., IL-1 β, TGF- β, PDGF, TNF-α, insulin growth factor-1, acid FGF, basic FGF) is upregulated [38, 70]. Chemokines produced by the infiltrated mononuclear cells may also play a critical role in chronic rejection processes. In the Lewis-to-F344 cardiac rat model, mRNA expression of the chemokines MCP-1, allograft inflammatory factor-1, and allograft inflammatory factor-2 is elevated [76, 92]. In this model, transplant arteriosclerosis is also associated with intragraft IFN-γ and IL-6 mRNA expression in the infiltrating cells.

Thus, analysis in animal transplant settings has shown that cytokines play key roles at every stage of the immune response after transplantation. In particular, the association between acute cellular rejection and the production of type 1 cytokines within the graft has prompted many investigators to analyze the role of these cytokines after clinical kidney, liver, and heart transplantation.

T-cell activation and the effect of immunosuppressive agents on cytokine production

The immune response after transplantation is largely governed by the actions of T-cells. Initiation of T-cell responses requires interaction of the T cell receptor with processed donor antigen, binding of CD4 and CD8 proteins with HLA class I and class II molecules, and secondary costimulatory signals between ligands on the T cell (CD28, CD40 ligand, CD2, LFA-1) and their counterparts (B7/CTLA-4, CD40, LFA-3, ICAM) present on antigen-presenting cells, including dendritic cells, Mf, B cells, and T cells [42, 49, 79, 82]. Not only cell surface events but also soluble mediators (IL-1, IL-6) contribute to T-cell activation [78]. The interactions trigger a number of intracellular events that lead to the production of cytokines and their receptors and to cellular proliferation. These intracellular events involve the activation of tyrosine kinases, tyrosine phosphorylation of cellular proteins followed by elevated, intracellular free calcium concentrations, and activation of the calcium- and phospholipid-dependent protein kinase C [14, 75]. This cascade leads to the initiation of transcription of cytokines and receptors. Transcription of cytokine genes is regulated by the binding of regulatory proteins (NF-AT, AP-1, OCT) to specific DNA sequences in the enhancer region of the gene [72]. The immunosuppressive action of cyclosporin A (CyA) and tacrolimus/FK506 is based on inhibition of the T-cell signal transduction pathways. In activated T cells, the rise in calcium activates the calmodulin-dependent phosphatase calcineurin. CyA and tacrolimus/FK506 inhibit calcineurin activity when it forms a complex with immunophilins, resulting in reduced cytokine production [33, 40].

In contrast to activation signals provided through the T-cell receptor, the effects of the CD28 costimulatory signals are not inhibited by CyA and tacrolimus/FK506 [99]. Glucocorticosteroids inhibit cytokine gene expression at multiple sites of the activation cascade. Steroids blocks cytokine production of T cells by inhibiting the IL-1 and IL-6 production of antigen-presenting cells, by inhibiting calcineurin-dependent pathways [65], and by interfering with the binding and/or transcriptional activity of NF-AT, OCT, and AP-1 of the IL-2 gene [65, 93]. In contrast, both rapamycin, although structurally very similar to tacrolimus/FK506, and mycophenolate mofetil have no effect on cytokine mRNA transcription [103]. Rapamycin blocks cytokine-driven T-cell proliferation by affecting proteins that are involved in cell cycle pathways and mycophenolate mofetil blocks the purine de novo synthesis that is required for DNA synthesis [15]. Another approach to inhibiting allogeneic immune responses is to block T-cell costimulation. A blockade of the CD28/B7 and/or CD40/CD40 ligand interactions results in prolonged survival of allografts, which can be associated with shifts in the type 1/type 2 balance [49, 68, 77]. Since cytokines appear to play major roles in transplant pathology, their receptors may also be good targets for selective immune therapy strategies.

Given the importance of IL-2 in the rejection process, monoclonal antibodies were developed to block the IL-2-dependent signalling pathway [44, 60, 84, 96, 97]. The high-affinity IL-2 receptor (IL-2R) consists of three transmembrane protein chains α, β, and γ. The α-chain is not expressed on resting T cells but is induced following activation. Moreover, the α-chain is necessary for the formation of the signal-transducing, high-affinity receptor [43]. Significant signal transduction components of cytokine receptors are members of the Janus family of kinases (JAKs) and signal transducers and activators of transcription (STATs). Cytokines may use common JAKs and STATs, which may, at least in part, explain phenomena of cytokine pleiotropy and redundancy [43]. For example, the γ-chain or the common γ_c subunit of the IL-2R is a component of high-affinity receptors for IL-2, IL-4, IL-7, IL-9, and IL-15, while the overlapping biological properties of IL-4 and IL-13 may be explained by another common receptor [17].

Thus, a blockade of cytokine receptors by monoclonal antibodies is a way of inhibiting cytokine-driven signal transduction pathways.

Interference at any level of the T-cell activation cascade by immunosuppressive agents is associated with changes in cytokine mRNA expression within the allograft. Therefore, monitoring of intragraft cytokine mRNA expression by RT-PCR is an ideal tool to determine the immune status of graft-infiltrating cells. This type of analysis may be helpful in treating patients more specifically with immunosuppressive therapies. However, extrapolation of in vitro or experimental data to the clinic demands caution. Interpretation of cytokine measurements may be hindered by all kinds of patient-related complications.

Intragraft cytokine measurements after clinical organ transplantation

Acute rejection

In transplantation immunology, shifts in the cytokine balance are often used to study mechanisms regulating acute rejection (type 1 response) or downregulation of the immune response (type 2 response). Consequently, most research in the clinical field have been focused on mediators produced by activated, infiltrating T lymphocytes (Table 1). In clinical kidney transplantation, the first evidence of a predominance of type 1 cytokines during the early stages of allograft rejection was reported by Dallman et al. [23]. In that report, intragraft IL-2 mRNA expression measured in fine needle aspirates preceded clinical rejection. Another study using fine needle aspirates also showed an association between intragraft type 1 (IFN-γ) cytokine mRNA expression and clinical acute rejection [61]. In contrast, Krams et al. did not find any relationship between IL-2 positivity and different stages of rejection [46]. They only occasionally found message coding for the IL-2 gene in solid biopsies from rejecting or rejected kidneys. Hutchinson's group was also unable to confirm an association between intragraft IFN-γ mRNA expression and kidney graft rejection [41]. Conflicting data on the involvement of type 1 cytokines were also reported in studies using in situ hybridization. Studies by Vandenbroeke et al. [95] and Grimm et al. [35] showed that neither IL-2 nor IFN-γ mRNA expression was associated with acute kidney allograft rejection, whereas Loong and colleagues found enhanced cytokine protein production of type 1 (IL-2, TNF-α) cytokines, their receptors (IL-2R), and type 2 (IL-4, IL-6, IL-10) cytokines in rejecting kidneys [52]. In line with this observation is the study by Xu et al. [102] in which intragraft expression of both type 1 (IL-2) and type 2 (IL-10) cytokines was upregulated during renal allograft rejection. Such a prominent role for type 2 cytokines in the rejection process after kidney transplantation was published by several groups. Krams et al. [46] found IL-4 and IL-5 mRNA expression in rejection biopsies and rejected kidneys, whereas Strehlau et al. [86] found heightened IL-10 mRNA expression in the absence of IL-4 mRNA.

Controversial data on monitoring type 1 cytokines during rejection have been reported not only in kidney transplantation but in clinical liver and heart transplantation as well. Indeed, several investigators have demonstrated that type 1 cytokines were detected or upregulated in rejecting livers [9, 13, 20, 29, 32] and hearts [11, 22, 34]; however, these and other studies also demonstrated that both hepatic and cardiac allograft rejection may occur in the absence of type 1 cytokines [48, 53, 105]. Thus, unlike the situation in animal models, the role of type 1 cytokines in the acute rejection process after clinical transplantation is less clear. From clinical studies it is obvious that allograft rejection may not only occur in the presence of both type 1 and type 2 cytokines but also in their absence. Yet, the presence of type 1 cytokines is nearly always associated with rejection and is downregulated again after successful antirejection treatment [10, 39]. IL-2 mRNA expression may be absent during immunological quiescence but may also simply indicate that the IL-2 mRNA signal disappeared during the rejection process. IL-2 is a gene that appears early in the course of an immune response, and Dallman showed that IL-2 mRNA expression can precede clinical rejection [21, 23].

Kinetics of IL-2 mRNA expression have shown that IL-2 mRNA is only briefly expressed by graft-infiltrating lymphocytes. After stimulation with donor antigen, IL-2 mRNA expression was detectable as early as 1–2 h post-activation and reached maximum levels between 2–48 h post-activation, and returned to baseline levels after 20–72 h [8]. Thus, timing is a factor that significantly complicates cytokine measurements. The IL-2 mRNA signal could easily have been missed.

Another explanation for IL-2-negative rejection is redundancy in the cytokine network. Recently, we found that proven blockade of the IL-2/IL-2R signalling pathway by CyA and an anti-IL-2R monoclonal antibody is not sufficient to prevent allograft rejection in cardiac allograft recipients [96]. Other cytokines may adopt the function of IL-2. For example, intragraft IL-7 and IL-15 (a T-cell growth factor secreted by macrophages) mRNA expression is present in rejected IL-2-negative renal and liver allografts and may well serve as an initiator of the allogeneic process [7, 67, 86].

Chronic rejection

Chronic allograft rejection is characterized by ongoing inflammation and diffuse concentric intimal prolifera-

Table 1 Intragraft cytokine mRNA profiles by RT-PCR associated with acute rejection after clinical organ transplantation (*K* kidney, *L* liver, *H* heart)

Organ	Cytokine profile(s) associated	Cytokine profile(s) not associated	References	Comments
K	IL-2		[23]	IL-2 mRNA expression preceded clinical rejection
K	IFN-γ		[61]	IFN-γ mRNA expression preceded clinical rejection
K	IL-4, IL-5, IL-6, TNF-α	IL-2, IFN-γ, IL-7	[46]	No comparison with either normal kidney tissue or biopsies without signs of rejection was made
K	IL-2 TNF-α, IL-6	IFN-γ, IL-4, IL-6, IL-10, TNF-α IL-2, IFN-γ, IL-4, IL-6	[41]	Early rejections (< day 100 post-transplantation) Late rejections (> day 100 post-transplantation)
K	IL-6	IFN-γ	[95]	Technique used: in situ hybridization
K	IL-2, IL-10	IFN-γ, IL-4, IL-7, TGF-β	[102]	Compared to chronic allograft nephropathy samples
K	IL-7, IL-10, IL-15	IL-2, IFN-γ, IL-4	[86]	Review article, see [74]
L	IL-2, IFN-γ, IL-6*, IL-1β*		[13]	Compared to "normal" non-transplanted liver tissue, * reduced levels
L	IL-1β, IL-2, IFN-γ, IL-6	IL-4, TNF-α	[20]	IL-2 only in early rejections
L	IL-2, IL-4, IL-5	IL-6, TNF-α	[29]	IL-5 only in the tacrolimus/FK506 group
L	IL-2	IL-4, IL-15	[7, 9]	Compared to biopsies both with and without histological signs of rejection
L	IL-2, IFN-γ	IL-4, IL-6, TGF-β	[32]	Compared to biopsies without evidence of rejection
L	IL-5	IL-2, IL-4, IL-6, IL-1β, TNF-α	[53]	Compared to biopsies without evidence of rejection
H	IL-2	IL-4, IL-10, IL-1β, TNF-α, TNF-β	[22]	In biopsies revealing severe rejection that required antirejection therapy
H	IL-2, IL-6	IL-4, IL-10	[6, 10, 11]	In biopsies revealing severe rejection that required antirejection therapy that inhibited the cytokine mRNA expression
H	IL-2	IFN-γ, IL-4, IL-6, IL-10, TNF-α	[34]	IL-2 only in early rejections
H	IL-6, TGF-β	IFN-γ, IL-4, IL-5, IL-1β, TNF-α	[105]	Only a trend for these cytokines was found
H	None	IL-1β, IL-2, IFN-γ, IL-4, IL-6, IL-10, TNF-α	[48]	No elevated cytokine mRNA levels were measured during a rejection period within the first 8 weeks post-transplantation
H	IL-2, IL-4, IL-5, IL-8, IL-10, TNF-α	IFN-γ, IL-3, IL-6, IL-9, IL-β, TNF-β	[98]	Technique used: in situ hybridization
H	IL-10		[5]	Technique used: in situ hybridization

Table 2 Intragraft cytokine mRNA profiles by RT-PCR associated with chronic rejection after clinical organ transplantation (*K* kidney, *L* liver, *H* heart)

Organ	Cytokine profile(s) associated	Cytokine profile(s) not associated	References	Comments
K	TGF-β		[81]	Compared to specimens with signs of acute rejection
K	IL-15	IL-2, IL-7	[86]	Review article; only in 2 out of 4 samples
L	IL-1β, IL-10	IL-2, IFN-γ, IL-6	[13]	Decreased cytokine mRNA expression compared to "normal" nontransplanted liver tissue
L	IL-2, IFN-γ, IL-5, PDGF	IL-4, IL-6, IL-8, IL-10, IL-1β, TNF-α	[37]	Compared to stable grafts
L	TGF-β		[26]	Technique used: in situ hybridization; compared to "normal" nontransplanted tissue
H	acidic FGF, PDGF		[104, 105]	Compared to "normal" nontransplanted tissue
H	RANTES		[66]	Technique used: in situ hybridization; compared to "normal" nontransplanted tissue
H	IL-2	IL-4, IL-6	[6]	The characteristics of the first acute rejection are associated with the diagnosis of chronic rejection at 1 year
		IL-2, IFN-γ, IL-4, IL-6, IL-10, TGF-β, PDGF		At time of diagnosis of chronic rejection at 1 year
H	basic FGF		[3]	Technique used: Northern blotting; compared to "normal" nontransplanted tissue

tion in the arterial system [12, 83]. This chronic process is thought to derive from an interaction between nonspecific and allogeneic factors, leading to smooth cell proliferation. The role of cytokines in the pathogenesis of transplant arteriosclerosis has been recognized for many years. The relationship between ischemia/reperfusion injury and the development of chronic rejection is well known although the underlying mechanism is poorly defined. Recently, Adams et al. showed that perfusion/ischemia leads to secretion of the chemokines macrophage inflammatory protein-1α and macrophage inflammatory factor-1 β by endothelial cells of the transplanted liver [1]. Chemokines produced by activated T cells and macrophages may also mediate the development of arteriosclerotic lesions. In contrast to what occurs in nontransplanted controls, the chemokine RANTES (regulated on activation normal T cell expressed and secreted) mRNA and protein production has been shown to be upregulated in coronary arteries of patients with end-stage chronic rejection after clinical heart transplantation [66]. Using in situ hybridization and immunohistochemistry, RANTES was localized in the infiltrating mononuclear cells and endothelial cells. In vitro studies showed that RANTES is produced by endothelial cells after exposure to TNF-α, IL-1β, or IFN-γ [27]. Therefore, routine intragraft chemokine measurements of time-zero and the first post-transplant biopsies may provide insight into how early chemokine production by endothelial cells or macrophages mediates mechanisms leading to acute and chronic rejection.

Apart from nonspecific factors such as ischemia/reperfusion injury, specific immune responses to the allograft can induce chronic inflammatory processes. This is based on the observation that a high incidence of acute rejection episodes is associated with the occurrence of chronic rejection. T-cell-derived cytokines such as IFN-γ may not only mediate the acute allograft response but also increase the expression of HLA and adhesion molecules and stimulate smooth muscle cell proliferation. However, evidence that graft arteriosclerosis is mediated by type 1 or type 2 cytokines is limited (Table 2). We found that production of type 1 cytokines preceded the diagnosis of chronic rejection after heart transplantation. Intragraft IL-2 mRNA expression during the first acute rejection episode and IFN-γ production by graft-infiltrating lymphocytes in the first 6 months post-transplant were associated with the early development of chronic rejection [6, 94]. In long-term transplanted grafts, endomyocardial lymphoid infiltration is common, but these cells do not transcribe detectable IL-2 or IL-4 mRNA, although mRNA expression of various growth factors was present [6]. This observa-

Table 3 Intragraft cytokine mRNA profiles by RT-PCR associated with graft acceptance after clinical organ transplantation (*K* kidney, *L* liver, *H* heart)

Organ	Cytokine profile associated	Cytokine profile(s) not associated	References	Comments
L	IL-10	IL-2, IFN-γ, IL-4, IL-6, IL-1β, IFN-γ	[20]	In stable grafts; IL-10 was absent during acute rejection
L	IL-4	IL-2, IL-15	[9]	In spontaneously resolving rejections
L	IL-4	IL-2, IFN-γ, IL-6, TGF-β	[32]	In the absence of histological and clinical signs of rejection
H	IL-10	IL-2, IFN-γ, IL-4, IL-6, TNF-α	[34]	Compared to biopsies showing severe rejection

tion suggests that cells producing type 1 cytokines are mainly involved in the initiation and not in the maintenance of transplant arteriosclerosis after heart transplantation. In liver grafts, an association between type 1 cytokines and end-stage chronic rejection was reported by Hayashi et al. [37]. However, intragraft analysis in renal biopsies showed that it is not type 1 cytokines but type 2 cytokines that are associated with graft arteriosclerosis [52, 55, 64].

These studies show that T cells producing both type 1 and type 2 cytokines are involved in the pathogenesis of chronic rejection. PDGF, basicFGF, insulin growth factor-1, TNF-α, and TGF-β are well-characterized growth regulators for endothelial cells and smooth muscle cells. An association between chronic rejection and the presence of one or more of these growth factors in the graft has been reported by several groups (Table 2). Messenger RNA expression of acidic FGF and basic FGF is upregulated in most transplanted hearts, irrespective of their chronic rejection state, implying that these mediators play a role in processes after transplantation; however, their specific involvement in the development of transplant arteriosclerosis has yet to be totally elucidated [3, 105]. The role of TGF-β, TNF-α, and PDGF in chronic rejection is somewhat clearer. In biopsies and specimens from grafts of patients who died of graft failure due to chronic rejection, the expression of TGF-β, TNF-α, and PDGF was found to be related to this complication [2, 26, 37, 52, 80, 81, 104]. However, interpretation of most of these data is limited since findings were compared to nontransplanted tissue instead of to transplanted organs without signs of chronic rejection. Moreover, cytokine production may be affected by the hypotensive period with warm ischemia during the terminal phase of life. Nevertheless, it may be assumed that increased production of TGF-β, TNF-α, and PDGF is of importance in the mediation of growth and repair mechanisms of smooth muscle cells and injured endothelial cells.

Cytokines and graft acceptance

Tolerance by donor-specific blood transfusion, a brief course of CyA, anti-CD4 mAb pretreatment, or a blockade of costimulatory signals is often associated ywith diminished type 1 cytokines and enhanced type 2 cytokines [16, 19, 77, 88]. These findings have raised the expectation that induction of a type 2 response to antigens might lead to donor-specific tolerance. However, the redundant and pleiotropic nature of the cytokine network suggests that induction and maintenance of transplant tolerance depend on complex mechanisms and cannot entirely be explained by the dichotomy into type 1/type 2 cytokines [69]. Recently, Strom et al. published "the traffic light" hypothesis for tolerance [87]. These authors postulate that tolerance induction can take place in the presence of autocrine IL-2 and IL-4 (green light), while in the presence of paracrine IL-2, IL-7, and IL-15 induction cannot be established (red light). Thus, in their opinion, it is not the nature of the cytokines that determines tolerance but the hierarchy of T-cell growth factors and their ability to mediate tolerance and rejection that determine cytokine production. After clinical transplantation, IL-4 mRNA expression is frequently measured during histopathological rejection and occasionally during immunological quiescence (Tables 1, 3) [9, 11, 20, 29, 32, 46, 53]. An indication that IL-4 may downregulate the immune response in patients was recently published. Spontaneously resolving liver graft rejection was found to be associated with intragraft IL-4 mRNA expression in the absence of IL-2 mRNA [8]. In line with this observation are the data published by Gorczynski and colleagues [32]. In a high proportion of liver biopsies obtained from patients without clinical evidence of rejection IL-4 mRNA expression was present in the absence of IL-2 mRNA expression. Kusaka et al. showed that after kidney transplantation, peripheral blood cells from a patient who discontinued all immunosuppressive drugs produced high amounts of IL-4 [47]. IL-10 is supposed to downregulate the donor-specific immune response as well. However, intragraft IL-10 mRNA expression was only occasionally associated with immunological quiescence.

IL-10 mRNA has been found to be present in hearts and livers with stable graft function and absent in rejecting grafts [20, 34]. However, most in vivo studies that have analyze intragraft IL-10 mRNA expression have not found any evidence of an immunosuppressive function. Instead, a positive correlation with acute rejection was observed in many cases [5, 86, 98,102]. The presence of type 2 cytokines within the graft during acute rejection can mean two things: either they are involved in the acute allograft response or, what is more interesting, they create an environment of immunological nonresponsiveness to the allograft. As such, production of type 2 cytokines may be the response of the immune system to type 1 cytokine-induced inflammatory responses, thereby restoring the balance of the cytokine network.

In order to further elucidate the role of type 2 cytokines in the clinical transplant setting, additional controlled studies need to be done. Analyses of serial biopsies of IL-4 and IL-10 mRNA expression in particular may provide information about the role of cells producing type 2 cytokines as down-regulators of specific immune responses. Another aspect of intragraft type 2 cytokines that would be interesting to study is their potential to monitor immunosuppression-weaning protocols. Such trials could provide much insight into the mechanisms of action of type 2 cytokines and their role in allograft acceptance.

Discussion

The current literature on cytokine production in clinical transplant settings raises as many questions as it answers. It shows no consensus on the type 1/type 2 (Th1/Th2) paradigm in transplantation (Tables 1–3). Yet, this is not entirely surprising given that data are generated from diverse clinical situations. Most of the studies differ from one another in terms of methodology, which makes it not always possible to compare data. The lack of association between rejection and immunological quiescence in the type 1/type 2 paradigm in human allograft responses is most likely due to the tremendous heterogeneity among allograft recipients and to the complexity of the cytokine network. Most biopsies after liver and kidney transplantation are taken in cases of deteriorating graft function, whereas after heart transplantation biopsies are taken on a routine basis. No doubt, the ways in which diagnoses are made strongly affect the interpretation of data regarding intragraft cytokine measurements. Consequently, it is difficult to differentiate which cytokines are involved in processes like rejection and graft acceptance. Nevertheless, these typically clinical complications do not preclude the likelihood of type 1 cytokines – IL-2 and IFN-γ – controlling the alloresponse to solid organ allografts and of type 2 cytokines – IL-4 and IL-10 – mediating responses associated with immunological quiescence or even tolerance. It has become evident that the type 1/type 2 paradigm is an oversimplified model that represents extremes of many possible outcomes [62]. Clones have been found to be capable of simultaneously producing both type 1 and type 2 cytokines [57]. Therefore, we assume that not only graft-infiltrating cells but also cells from the graft itself can produce type 1 and type 2 cytokines during an immune response. We are currently aware of pleiotropism and redundancy of the cytokine network. Potent cyclosporin-resistant T-cell growth factors such as IL-7 and IL-15 mRNA are present rejected in IL-2-negative grafts, which suggests that these mediators participate in the rejection process [7, 13, 86]. Redundancy of the cytokine network also implies that elimination of a single mediator of the cytokine cascade may not be enough to inhibit an allogeneic response. Clinical data can only be interpreted in the context of the specific immunosuppressive drugs used, as those agents may interfere with local cytokine production. Several reports suggest that agents that block the calcineurin pathway selectively inhibit type 1 cytokines while sparing type 2 cytokine production [28, 71]. This may have great clinical implications; however, there are no clinical studies to support these data. The association between type 1 cytokines and acute rejection is often based on timing. In both animal and human studies, intragraft IL-2 mRNA expression may precede the rejection episode [23, 24, 54]. However, this early T-cell activation marker is not specifically associated with clinically significant rejection. Type 1 cytokines are measured in early serial fine needle aspirates obtained from rejecting as well as from nonrejecting infiltrates, probably reflecting a common inflammatory response due to surgical trauma, ischemia, or reperfusion injury [54]. Moreover, the presence of a particular cytokine at the graft site is not direct evidence of its participation in the rejection response. The fact that local production of type 1 cytokines alone is not sufficient for the development of acute rejection suggests that not all elements are available for an effective immune response. Unknown cytokines or other components of the immune system may be involved. Even cytokines produced by the graft itself may control the specific immune response.

For chronic rejection, the situation is even more complicated. The etiology of chronic rejection is multifactorial and can be divided into immunological and nonimmunological factors. For example, oxidatively modified low-density lipoproteins may stimulate secretion of MCP-1 and colony-stimulating factors by cultured endothelial cells, resulting in enhanced monocyte adherence to these cells [50]. This nonspecific trigger of cytokine production may also have consequences for acute allogeneic responses, thereby accelerating the development of transplant arteriosclerosis.

Despite the overwhelming amount of literature on cytokine measurements in clinical transplant settings, little, if any, attention has been paid to the analysis of cytokine mRNA expression in biopsies taken 5–10 years after transplantation. Such an analysis could shed some light on the mechanisms involved not only in chronic allograft rejection but also in graft acceptance.

Recently, a new approach to cytokine analysis using PCR applications was reported by Hutchinson and colleagues [91]. They correlated presence of polymorphic microsatellite markers in the TNF-*a* gene with cytokine production in vitro. It was found that heart transplant recipients with the microsatellite TNFd3 produced significantly more TNF-α than TNFd3-negative patients. A mutation at position –308 in the promotor region of the TNF-α gene is also associated with increased TNF-α production, while the presence of an A at position –1082 in the IL-10 gene is correlated with decreased IL-10 production [90, 100]. Analysis of these cytokine genotypes showed that heart transplant recipients typed as high TNF-α and low IL-10 producers had significantly more severe acute rejection episodes than patients typed as low TNF-α and high IL-10 [90]. This raises the possibility of using cytokine genotypes as a marker to identify pretransplantation which patients are likely to reject their allografts and which patients are likely to accept them. From this perspective, both donor and recipient genotype are important.

Conclusions

In summary, this review shows that data gained from experimental studies on the cytokine network are, at best, only an indication of immune processes in clinical transplantation. The many reports on cytokine measurements in transplant recipients have produced a tremendous amount of conflicting data. Nevertheless, it is possible to characterize specific cytokine patterns within the graft during acute and chronic rejection and graft acceptance. Although cytokine profiles may vary between heart, kidney, and liver allograft recipients, cytokine measurements are extremely useful in monitoring the efficacy of immunosuppressive drugs and in characterizing changes in cytokine profiles in individual patients. They may enable us to identify patients who require more or less, or even no, immunosuppressive therapy. The link between cytokine polymorphisms and cytokine production is especially promising and could open the way to prospective immunological typing in clinical transplantation.

Until the mechanisms of action that control cytokine activity are clarified, we have to exercise caution with regard to the clinical significance of intragraft cytokine gene expression in transplantation. To elucidate the precise function of cytokines in humans, additional controlled studies are needed. Monitoring of therapeutic and cytokine intervention trials will help us to unravel the complex cytokine network that should eventually lead to better care and treatment of transplant recipients.

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