EXTENDED REPORT

Preferential type 1 chemokine receptors and cytokine production of CD28⁻ T cells in ankylosing spondylitis

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Objective: To examine serum levels of type 1 and type 2 chemokines and lymphocytic expression of chemokine receptors, and to compare the results with lymphocytic cytokine production in patients with ankylosing spondylitis (AS).

Methods: Twelve patients with AS (mean (SD) age 44.9 (14.7) years) and 27 healthy controls (46.4 (12.8) years) were enrolled into the study. The expression of chemokine receptors (CCR-5, CXCR-3, CCR-4) and cytokines (interferon γ (IFN γ), interleukin (IL)2, IL4, IL10, tumour necrosis factor α (TNF α)) on CD28⁺ and CD28⁻ T cell subtypes was analysed by a three colour FACS technique of peripheral blood samples. Serum ELISAs were performed to detect the CCR-5 ligands CCL-5, CCL-3; the CXCR-3 ligands CXCL-10, CXCL-9; and the CCR-4 ligand, CCL-17 before and after administration of the TNF α blocking agent infliximab.

Results: CD4⁺CD28⁻ T cells had higher ratios of CXCR-3 to CCR-4 than CD4⁺CD28⁺ T cells. Both, CD4⁺ and CD8⁺CD28⁻ T cells of patients with AS produced more IFN γ , TNF α , and IL10 than their CD28⁺ counterparts ($p < 0.05$), and lacked the production of IL2 and IL4. Serum levels of CXCL-9 were increased in patients with AS to 59.2 pg/ml (34.1–730.5) compared with 32.5 pg/ml (20.0–79.5) in healthy controls ($p = 0.016$). The levels of both type 1 (CCL-5, CXCL-9) and type 2 chemokines (CCL-17) decreased under blockade of TNF α ($p < 0.05$).

Conclusions: The profile of chemokine receptor expression and cytokine production by CD28⁻ T cells suggests a type 1 immune reaction in AS, although IL10 is frequently produced by CD28⁻ T cells. Treatment with TNF α blocking antibodies decreased both types of chemokines in patients' sera.

Polarisation and heterogeneity of T cells is considered to be important in the initiation and perpetuation of synovial inflammation, and the model of type 1 and type 2 immune responses has attracted great interest in immune mediated diseases.¹ In spondyloarthritis (SpA), including ankylosing spondylitis (AS), however, cytokine profiles seem to be more complicated than in other diseases.² A few studies examined cytokines in patients with SpA, with divergent conclusions about the type of the immune reactions. Several investigators described an increment of proinflammatory cytokines (interleukin (IL)6, interferon γ (IFN γ)) and the anti-inflammatory cytokine IL10 in active SpA and psoriatic arthritis.^{3–5} Others suggested a Th2 cytokine profile in SpA with relatively little amounts of IFN γ and tumour necrosis factor α (TNF α) and increased cytokine mRNA of IL10 in synovial fluid compared with patients with rheumatoid arthritis (RA).⁶ At the cellular level Th1 cytokine production appeared to be lower in patients with SpA than in healthy controls.^{7–8}

The strong association between HLA-B27 and AS suggests an important role not only for CD8⁺ cytotoxic T cells but also for CD4⁺ T helper cells in this disease.^{9–10} Indeed, immunohistological studies of sacroiliac biopsy specimens showed dense cellular infiltrations of T cells and macrophages and massive expression of TNF α within the synovial part of the sacroiliac joints.¹¹ In patients with SpA treated with TNF α blocking agents peripheral blood mononuclear cells (PBMCs) stimulated with phorbol 12-myristate 13-acetate (PMA)/ionomycin produced either higher or lower levels of Th1 cytokines.^{2–12–13} Recently, our own group described peripheral enrichment of proinflammatory IFN γ -producing CD4⁺ and CD8⁺ T cells lacking the costimulatory molecule CD28 in

patients with AS, suggesting a role for these cells in a type 1 immune response, although other markers have not been tested so far.^{14–15} Such CD28⁻ T cells occur in many chronic inflammatory disorders including RA, Wegener's granulomatosis, and multiple sclerosis,^{16–18} and are considered as markers for chronic inflammation and early aging.¹⁹ Under these circumstances the CD28⁻ T cells are part of the CD4⁺ as well as the CD8⁺ T cell compartment, persist over years, and include most of the oligoclonally expanded T cells. Phenotypically, CD4⁺CD28⁻ T cells from patients with RA and AS and CD8⁺CD28⁻ T cells from elderly people, and patients with melanoma share the expression of various NK cell receptors, and lack expression of the lymphocyte marker CD7.^{20–22} Functionally these T cells can release large amounts of IFN γ , perforin, and granzyme B, making it possible for them to lyse target cells.²³ In patients with AS the prevalences of both CD4⁺ and CD8⁺CD28⁻ T cells depend on the disease status.^{14–15}

Chemokines are directly involved in the extravasation of T cells into inflamed tissue. Expression of a specific chemokine receptor profile on lymphocytes was described as an additional marker for the immune response in various immune mediated diseases. Typical type 1 T cells show strong surface expression of CCR-5 and CXCR-3 and produce

Abbreviations: AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IFN γ , interferon γ ; IL, interleukin; PBMCs, peripheral blood mononuclear cells; PMA, phorbol 12-myristate 13-acetate; RA, rheumatoid arthritis; SpA, spondyloarthritis; TNF α , tumour necrosis factor α .

Table 1 Overview of chemokine receptors, chemokines with alternative nomenclature in parentheses, and the corresponding immune response.

Chemokine receptor	Chemokine	Immune response
CCR-5	CCL-5 (RANTES), CCL-3 (MIP-1 α)	Type 1
CXCR-3	CXCL-9 (MIG), CXCL-10 (IP-10)	Type 1
CCR-4	CCL-17 (TARC)	Type 2

high concentrations of IL2 and IFN γ .^{24–25} The lymphocytic type 2 response is characterised by high expression of CCR-3, CCR-4, and CCR-8, combined with increased production of IL4, IL5, IL6, IL10, and IL13.^{26–27} Table 1 provides an overview of the chemokine receptors examined, their ligands, and the corresponding immune response.

The aim of this study was to examine the expression of both type 1 and type 2 chemokine receptors on CD28⁺ and CD28[−] T cells and to compare them with serum chemokine levels and the intracellular production of cytokines in T cells from patients with AS.

PATIENTS AND METHODS

Patients

Twelve consecutive patients (three female, mean (SD) age 44.9 (14.7) years) with definite AS according to the modified New York criteria²⁸ were recruited into the study. Patients with current pregnancy or lactation, a history of neoplasm, recent acute infection, or history of any other chronic inflammatory disease were excluded from the study. The same exclusion criteria were applied for 27 healthy controls matched for age and sex (six female, age 46.4 (12.8) years). After informed and written consent had been received from the subjects, clinical and laboratory measures were routinely assessed and peripheral blood was drawn for further analyses as approved by the local ethics committee.

Table 2 shows the patients' characteristics, including the Bath Ankylosing Spondylitis Metrology Index (BASMI),²⁹ the Bath Ankylosing Spondylitis Functional Index (BASFI),³⁰ and the Bath Ankylosing Disease Activity Index (BASDAI).³¹ A subgroup of six patients with active AS disease were treated with chimeric anti-TNF α monoclonal antibodies (infliximab (Remicade), Schering Plough/Aesca, Vienna, Austria) at a dosage of 3 mg/kg body weight. With treatment the BASDAI improved from 6.2 (1.9) to 3.2 (2.0), erythrocyte sedimentation rate (ESR) from 36.8 (19.5) mm/1st h to 11.2 (3.0) mm/1st h, and C-reactive protein (CRP) levels from 23 (2.0) mg/l to 4 (2) mg/l (shown as mean (SD)).

Enzyme linked immunosorbent assays

Enzyme linked immunosorbent assays (ELISAs) were performed in duplicate to determine concentrations of CCL-5 (RANTES), CCL-3 (MIP-1 α), CXCL-9 (MIG), CXCL-10 (IP-10), and CXCL-17 (TARC), according to the manufacturer's

instructions (R&D Systems, Inc, MN, USA), in blinded serum samples.

Surface staining for chemokine receptors

To determine the peripheral level of CD3⁺CD4⁺CD28[−] and CD3⁺CD8⁺CD28[−] T cells, the expression of chemokine receptors, and intracellular cytokine production of CD4⁺ and CD8⁺CD28[−] T cells, PBMCs were isolated by Ficoll density gradient centrifugation. Surface staining of PBMCs was performed using fluorescein isothiocyanate conjugated anti-CCR-5 (R&D Systems, Inc, Minneapolis, MN, USA), anti-CD28, anti-CD4, and anti-CD8; phycoerythrin conjugated anti-CD28, anti-CXCR-3, and anti-CCR-4; and peridinin chlorophyll protein conjugated anti-CD4, anti-CD8, and anti-CD3 monoclonal antibodies (all from Becton Dickinson, San Diego, CA, USA).

Intracellular staining for cytokine production and flow cytometry

For intracellular staining, cells were stimulated with 25 ng/ml PMA and 1 μ g/ml ionomycin in the presence of 10 μ g/ml brefeldin A for 4 hours (Sigma, Munich, Germany). After cell surface staining for CD4 and CD28 with subsequent fixation and permeabilisation, cells were stained with fluorescein isothiocyanate conjugated anti-IFN γ , anti-TNF α , anti-IL2, anti-IL4, anti-IL10 antibodies or control immunoglobulin (Becton Dickinson).

After final fixation with 1% cell fix (Becton Dickinson), cells were analysed on a FACS-Calibur flow cytometer (Becton Dickinson). Data were analysed using WinMDI software (version 2.8; Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA).

Statistics

Results were tested for the distribution using the Kolmogorov-Smirnov test and expressed as median and range. The Mann-Whitney test was used to compare the independent groups, and the Wilcoxon ranking test to compare paired data from the CD28⁺ and the CD28[−] T cell compartments. The Spearman correlation (r_s) test was performed to examine possible correlations. All statistical analyses were performed using the SPSS program, version 11.5 (Chicago, IL, USA). A value of $p < 0.05$ was considered significant.

RESULTS

The prevalences of CD3⁺CD4⁺CD28[−] and CD3⁺CD8⁺CD28[−] T cells in patients with AS were increased to 2.3% (0.9–8.8%) and 38.6% (10.4–52.9%) in comparison with 0.6% (0.1–1.2%) and 24.5% (7.6–37.4%) in healthy controls ($p < 0.001$, $p = 0.037$, respectively).

Differential surface expression of chemokine receptors on CD28⁺ and CD28[−] T cells

Surface expression of both type 1 (CCR-5, CXCR-3) and type 2 (CCR-4) chemokine receptors was examined by FACS analysis, and compared between the CD28⁺ and the CD28[−] T cell subsets from patients with AS and healthy controls. Figure 1 shows that the expression of chemokine receptors differed markedly between CD28⁺ and CD28[−] T cells, both on CD4⁺ and CD8⁺ T cells from patients with AS and healthy controls. CD4⁺CD28[−] T cells were not detectable in healthy controls. CCR-5 expression was low on all subtypes of T cells. However, CXCR-3 was similarly expressed on CD4⁺CD28⁺ and CD28[−] T cells, but more frequently on CD8⁺CD28⁺ than on CD28[−] T cells (22.7% (5.7–28.7) v 2.4% (0.2–88.9) positive cells, $p = 0.010$). The CCR-4 receptors were expressed more frequently on CD4⁺CD28⁺ than on CD28[−] T cells (41.1% (25.0–62.5) v 4.0% (0.7–6.0) positive cells, $p = 0.001$), and

Table 2 Patients' characteristics

Characteristic	
HLA-B27 positivity (%)	92
BASMI	5.1 (2.5)
BASFI	4.3 (2.3)
BASDAI	6.7 (1.9)
ESR (mm/1st h)*	31.8 (21.3)
C reactive protein (mg/l)†	20 (16)

Results are shown as mean (SD).

*Normal < 15 mm/1st h; †normal < 7 mg/l.

Figure 1 Expression of the chemokine receptors CCR-5 (A), CXCR-3 (B), CCR-4 (C), and the calculated ratio of CXCR-3 to CCR-4 (D) on CD4⁺ T cells and CCR-5 (E), CXCR-3 (F), CCR-4 (G) and the calculated ratio of CXCR-3 to CCR-4 (H) on CD8⁺ T cells from the peripheral blood of healthy controls (Co) and patients with AS (AS). Whiskers box plots show 50% of cases within the boxes and all data excluding mavericks between the end points of the whiskers (lines). $p < 0.05$ is considered significant (using the paired Wilcoxon ranking test).

Figure 2 Expression of the chemokine receptors CCR-5 (A), CXCR-3 (B), CCR-4 (C), and the calculated ratio of CXCR-3 to CCR-4 (D) on CD4⁺ T cells and CCR-5 (E), CXCR-3 (F), CCR-4 (G), and the calculated ratio of CXCR-3 to CCR-4 (H) on CD8⁺ T cells from the synovial fluid of four patients with AS (AS). The percentages of CD28⁺ and CD28⁻ T cells positively stained for the chemokine receptor are shown for each patient. $p < 0.05$ is considered significant (using the paired Wilcoxon ranking test).

only on CD8⁺CD28⁺ from patients with AS compared with CD28⁻ T cells (12.3% (3.2–76.8) *v* 0.8% (0.5–1.6) positive cells, *p* < 0.001). Taken together, CD4⁺CD28⁻ T cells had a higher ratio of CXCR-3/CCR-4 than CD4⁺CD28⁺ T cells (12.3 (3.2–76.8) *v* 0.8 (0.5–1.6), *p* < 0.001) (fig 1D). In healthy controls, however the CXCR-3/CCR-4 ratio from controls was higher for the CD8⁺CD28⁺ than for the CD8⁺CD28⁻ T cells (6.9 (5.1–30.3) *v* 0.4 (0.0–32.1), *p* = 0.002). No correlation was detected between the expression of chemokine receptors on CD4⁺ and CD8⁺ T cells and the clinical measures (BASMI, BASFI, BASDAI) or the serological variables (ESR and CRP levels).

Synovial fluid samples were available from four patients who underwent therapeutic joint puncture. In the synovial fluid, 1.6% (0.1–6.0) of the CD4⁺ T cells and 12.2% (6.4–20.5) of the CD8⁺ T cells lacked the costimulatory molecule CD28. As in the peripheral blood CD4⁺CD28⁺ and CD4⁺CD28⁻ T cells, but also CD8⁺CD28⁺ and CD8⁺CD28⁻ T cells showed equal expression of CXCR-3. The expression of CCR-4 was more frequent on CD28⁺ than on CD28⁻ cells. The expression of CCR-5 was low on CD4⁺ and CD8⁺ T cell subsets. The CXCR-3/CCR-4 ratio was calculated to be 13.3 (10.9–15.8) for CD4⁺CD28⁻ T cells *v* 3.1 (0.7–5.9) for CD4⁺CD28⁺ (*p* = 0.064) and 56.6 (48.8–64.4) for CD8⁺CD28⁻ T cells *v* 12.2 (2.1–39.4) for CD8⁺CD28⁺ (*p* = 0.021) (figs 2A–H).

Comparison of intracellular cytokine profiles of CD28⁻ and CD28⁺ T cells

As the observed chemokine receptor expression would argue for a type 1 immune response of fresh CD28⁻ T cells in AS compared with their CD28⁺ counterparts, the T cell subsets were further tested for their intracellular production of type 1 and type 2 cytokines after prior stimulation with PMA/ionomycin in the presence of brefeldin A.

From this functional perspective, production of the proinflammatory cytokines IFN γ , TNF α , and the anti-inflammatory cytokine IL10 were more frequent in both the CD4⁺ and the CD8⁺CD28⁻ T cells, compared with their CD28⁺ counterparts. IL2 production was less frequent in CD4⁺ and CD8⁺CD28⁻ T cells than in their CD28⁺ T cell counterparts. IL4 production was low in all CD4⁺ and CD8⁺ T cells (figs 3A–I). No differences in cytokine production were detected between CD28⁺ and CD28⁻ T cells from controls and patients with AS (data not shown).

Serum levels of chemokines

Expression of chemokine receptors on proinflammatory CD4⁺ and CD8⁺ T cells of patients with AS suggested a role for chemokines in AS. Therefore we tested the serum levels of typical type 1 and type 2 chemokines, and compared them between healthy controls and patients with AS (figs 4A–E). Serum levels of CXCL-9, a ligand of the type 1 CXCR-3 receptor, were increased in patients with AS to 59.2 pg/ml (34.1–730.5) compared with 32.5 pg/ml (20.0–79.5) in healthy controls (*p* = 0.016). In contrast the serum levels of the CCR-5 ligands, CCL-5 and CCL-3; the other CXCR-3 ligand tested, CXCL-10; as well as the CCR-4 ligand, CCL-17 showed no significant differences between patients with AS and healthy controls. Although, a strong correlation was detected between serum levels of the type 1 chemokine CCL-5 and ESR levels of patients with AS (*r*_s = 0.725, *p* = 0.025 using the Spearman correlation test), correlations between the other serum chemokines, laboratory and clinical variables tested were negative.

Effects of TNF α blocking treatment on chemokine receptor expression and serum chemokine levels

The expression of type 1 chemokine receptors on CD28⁻ T cells and the increased serum levels of the CXCR-3 receptor

Figure 3 Production of IL2 (A), IFN γ (B), TNF α (C), IL4 (D), IL10 (E) in CD4⁺ T cells and IL2 (F), IFN γ (G), TNF α (H), IL4 (I), IL10 (J) in CD8⁺ T cells after stimulation with PMA/ionomycin. Whiskers box plots show 50% of cases within the boxes and all data excluding mavericks between the end points of the whiskers (lines). *p* < 0.05 is considered significant (using the paired Wilcoxon ranking test).

Figure 4 Chemokine levels of the CCR-5 ligands, CCL-5 (A) and CCL-3 (B), the CXCR-3 ligands, CXCL-10 (C) and CXCL-9 (D), and the CCR-4 ligand CXCL-17 (E) in healthy controls and patients with AS. Whiskers box plots show 50% of cases within the boxes and all data excluding mavericks between the end points of the whiskers (lines). $p < 0.05$ is considered significant (using the Mann-Whitney test). After blockade of $\text{TNF}\alpha$, the serum levels of CCL-5 (F), CXCL-10 (H), and CCL-17 (J) decreased, whereas the serum levels of CCL-3 (G) and CXCL-9 (I) showed no differences before and after treatment. The serum levels before and after treatment with infliximab are marked by a filled circle for each single patient. $p < 0.05$ is considered significant (using the paired Wilcoxon ranking test).

ligand, CXCL-9, raised the question whether $\text{TNF}\alpha$ blocking agents would affect the chemokine system. Retesting during successful $\text{TNF}\alpha$ blocking treatment showed that the expression of both the CXCR-3 and the CCR-4 receptors was unchanged on CD4^+ and CD8^+ independently of their CD28 subtype.

The serum levels of CCL-5, CXCL-10, and CCL-17 decreased under successful blockade of $\text{TNF}\alpha$, whereas the serum levels of CCL-3 and CXCL-9 showed no differences before or after treatment. Thus both type 1 and type 2 chemokines were affected by blockade with $\text{TNF}\alpha$ antibodies (figs 4F–J).

DISCUSSION

According to this study lymphocytic expression of chemokine receptors and intracellular cytokine production preferentially show signs of a type 1 immune response on CD28^- T cells from patients with AS. Thus assessment of the chemokine receptor profile to describe the type of immune response in AS has supported the cytokine production data available so far.^{14, 15} In particular, expression of the CXCR-3 receptor was increased on $\text{CD4}^+\text{CD28}^-$ T cells of patients with AS, while the expression of the other type 1 chemokine receptor, CCR-5, was low on both CD28^+ and CD28^- T cells, even though CD28^- T cells were described as frequently expressing CCR-5, for instance in patients with Wegener's granulomatosis.^{32, 33} As the expression of CXCR-3 on peripheral CD4^+ T cells is largely restricted to the memory population,³⁴ this is consistent with the described memory-effector phenotype of $\text{CD4}^+\text{CD28}^-$ T cells.

Others had already demonstrated an increased expression of CCR-4 on circulating CD4^+ T cells in patients with AS compared with healthy controls and a correlation between the percentages of $\text{CD4}^+\text{CCR-4}^+$ T cells with AS disease status measured by the BASDAI score.³⁵ In our study, there was a preferential expression of CCR-4 on $\text{CD8}^+\text{CD28}^+$ T cells of

patients with AS compared with $\text{CD8}^+\text{CD28}^+$ T cells of healthy controls. CD4^+ and $\text{CD8}^+\text{CD28}^+$ T cells of patients with AS expressed CCR-4 more frequently than their CD28^- T cell counterparts. Expression of both CXCR-3 and CCR-4 did not correlate with disease activity measured by the BASDAI score (data not shown).

A recent study demonstrated an increased expression of CXCR-3 on memory-effector T lymphocytes in patients with inflammatory liver diseases compared with lymphocytes of healthy livers and the peripheral blood from healthy controls.³⁶ Also in our study, the expression of chemokine receptor CXCR-3 was increased on both, CD4^+ and CD8^+ subpopulations of T cells from the synovial fluid. This expression pattern resulted in an increased CXCR-3/CCR-4 ratio in $\text{CD4}^+\text{CD28}^-$ and $\text{CD8}^+\text{CD28}^-$ T cells compared with $\text{CD4}^+\text{CD28}^+$ and $\text{CD8}^+\text{CD28}^+$ T cells and may reflect a more activated immune status at the site of inflammation compared with the peripheral blood. Because of the increased prevalence of circulating CD4^+ and $\text{CD8}^+\text{CD28}^-$ T cells in AS and their preferential expression of the type 1 chemokine receptor CXCR-3 in the peripheral blood as well as the synovial fluid, these memory-effector CD28^- CXCR-3⁺ T lymphocytes can be expected to have a role in AS. The presence of these cells supports the concept of a disease with a precise antigenic target as a stimulus for disease progression which, however, is still unknown for AS.

Interestingly, stimulated fresh CD28^- T cells produced more of the type 2 cytokine IL10 than their CD28^+ counterpart. This observation is in accordance with increased plasma levels of IL10 in patients with AS, which even correlated with disease activity. Also for RA, IL10 has been discussed as a possible regulator of the immune dysfunction and correlated with progression of joint destruction.³⁷ On the other hand, IL10 is known to be an immunosuppressive cytokine and may be increased in an attempt to counterbalance the

pathological proinflammatory immune status in AS disease. Thus both type 1 and type 2 cytokines are produced in stimulated CD28⁺ T cells from patients with AS despite a clear type 1 chemokine receptor profile of these cells.

It has been suggested that TNF α has a central role in many immune mediated diseases, including AS.³⁸ Under clinically successful blockade of TNF α , we found no effect on the expression of CXCR-3 or CCR-4 on CD28⁺ T cells (data not shown), whereas a sustained accumulation of circulating CXCR-3 positive T lymphocytes had been reported using TNF α blocking agents in patients with RA.³⁹ It has been shown earlier that TNF α blockade down regulates both IFN γ and TNF α secreted by T cells but does not induce a change in cytokines produced by monocytes during 3 months of treatment.¹² In our short term study the chemokine levels of CCL-5, CXCL-10, and CCL-17 were reduced under blockade of TNF α , whereas the serum levels of CCL-3 and CXCL-9 showed no differences before and after treatment. These results may reflect the complex mechanism of effect and counter-effect of chemokines involved in the pathogenesis of AS, but these data have to be confirmed in a larger cohort of patients with AS.

The involvement of chemokines in the pathophysiology of AS opens a new perspective for possible therapeutic approaches. Both chemokines released from the inflamed synovial cells and chemokine receptors expressed on cells infiltrating the synovial tissue may serve as a new therapeutic target not only in RA but also in AS.⁴⁰ We know already that antibody mediated blockade of the CXCR-3 chemokine receptor reduced recruitment of T helper 1 cells into sites of inflammation in an adjuvant-induced peritonitis model.⁴¹ The in vivo neutralisation of CXCL-9 or CXCL-10 reduced the severity of idiopathic pneumonia syndrome in a murine model compared with control treated animals; an additive effect was observed when both ligands were blocked simultaneously.⁴²

In conclusion, the chemokine CXCL-9 is increased in sera from patients with active AS compared with healthy controls, and CCL-5, CXCL-10, and CCL-17 decrease during successful treatment with a TNF α blocking agent. The expression profile of chemokine receptors on T cells reflects the proinflammatory, IFN γ -producing type of CD28⁺ T cells, whereas production of type 2 cytokines cannot be totally neglected.

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