Increased γ/δ-positive T-cells in blood and bronchoalveolar lavage of patients with sarcoidosis and hypersensitivity pneumonitis

M. Rauf*, V. Liebers*, C. Steppert**, X. Baur*


ABSTRACT: A small population of T-cells does not express the conventional T-cell receptor (TCR), characterized by the α and β polypeptide chains (α/β TCR) but two polypeptides termed γ and δ (γ/δ TCR). Changes in γ/δ TCR expression may be relevant as the cause or consequence of several diseases. Our study was undertaken to determine and compare the distribution of T-cells expressing γ/δ TCR in blood and bronchoalveolar lavage (BAL) of patients with sarcoidosis, hypersensitivity pneumonitis (HP), idiopathic pulmonary fibrosis (IPF), and of healthy controls. In addition, the association between γ/δ TCR of blood T-lymphocytes and accessory molecules was analysed.

Using direct immunofluorescence with the anti-γ/δ TCR and anti-CD3 monoclonal antibodies (MoAbs) followed by flow cytometric analysis, the blood of patients with pulmonary sarcoidosis, HP, IPF and of healthy controls was analysed. To reveal the association between γ/δ TCR of blood T-lymphocytes and the accessory molecules, expression of CD4, CD8 and CD25 were determined.

Calculating the percentage and the total number of CD3+ γ/δ TCR cells in blood, the data indicated a significant increase of γ/δ T-cells in individuals with pulmonary sarcoidosis and HP, compared to healthy controls and IPF patients. In sarcoidosis patients with elevated CD3+ γ/δ TCR levels, significantly lower CD4/CD8 ratios were observed. In addition, our data demonstrate a correlation between the decrease of CD4+ cells in blood and the amplified appearance of γ/δ TCR expression in sarcoidosis patients, but not in HP patients. γ/δ TCR+ cells were either CD8-positive (approximately 50%), or had a "double-negative phenotype" (CD4−/CD8−) (remaining 50%). Circulating blood γ/δ T-cells were CD25-negative, suggesting a lack of activation. The total number of CD3+ γ/δ TCR+ bearing lymphocytes in BAL fluid was increased in sarcoidosis and HP, compared with IPF. A corresponding increase of CD3+ γ/δ TCR+ cells was observed in blood and BAL of sarcoidosis patients.

In conclusion, our data indicate that levels of γ/δ T-cells are increased in blood and BAL fluid of patients with sarcoidosis and HP. Although this suggests a pathogenetic role for γ/δ TCR, it is not clear whether its expression is a causal factor, or a consequence, of these diseases.

Eur Respir J., 1994, 7, 140–147.

The T-cell antigen receptor (TCR), a heterodimer closely associated with the CD3 complex on cell surfaces, and composed of α- and β-chains, is expressed on most T-cells. Normally, antigens are recognized by α/β T-cells, as short peptides presented by major histocompatibility complex (MHC) molecules. Another type of recognition occurs with bacterial superantigens, which bind to the microbiological regions of class II MHC molecules [1]. These superantigens activate αβ T-cells, which express particular Vβ gene products, irrespective of their nominal antigenic specificity in an MHC-unrestricted manner. A minor population of T-cells, bearing an antigen receptor which is composed of γ and δ subunits was recently identified [2]. The nature of the antigens recognized by γ/δ T-cells, and the mode of their presentation, are still largely unknown. A subgroup of γ/δ T-cells, ex-pressing TCR by using Vγ9 and Vδ2 gene product pairs, responds to mycobacterial antigens, representing a superantigen-specific phenomenon [3]. Changes in γ/δ TCR expression may be relevant as the cause or consequence of several diseases. γ/δ T-cells are increased in some infectious diseases, e.g. localized cutaneous leishmaniasis, reversal
reactions in leprosy, the area surrounding zones of necrosis in tuberculous lymphadenitis, and the peripheral blood of patients with measles, and in immunological disorders such as rheumatoid arthritis and coeliac disease [2].

Sarcoidosis is a chronic, nonmalignant, multisystem disease, often affecting the lungs. The appearance of T-lymphocytes, especially CD4-positive T-lymphocytes, can be demonstrated in increased numbers by bronchoalveolar lavage (BAL) [4]. Studies to understand the pathogenesis of this disease have focused on evaluating the T-cell activation, as a result of antigen interaction with the TCR [5]. Increased numbers of γδT-lymphocytes in BAL and blood have been reported in a subgroup of patients with sarcoidosis [6].

Hypersensitivity pneumonitis (HP), also called extrinsic allergic alveolitis (EAA), is a chronic inflammatory lung disease [7, 8], due to a wide variety of environmentally and occupationally encountered organic dusts (e.g. spores from bacteria or saprophytic fungi, bird antigens), and some chemicals (e.g. isocyanates). Recent studies have reported that HP was associated with an influx of CD8+ T-lymphocytes with cytotoxic suppressor function and macrophage activation in the BAL [9, 10]. The reason why HP only develops in some exposed persons is unknown. However, whilst specific antibodies occur in the blood of both exposed asymptomatic persons and in affected patients, specifically sensitized T-lymphocytes seem to occur more frequently in patients. This finding suggests that T-cell mediated, delayed hypersensitivity mechanisms are involved in the pathogenesis [11].

Sarcoidosis and HP are two different interstitial lung disorders, with specific pathogenic mechanisms and characteristics. Little is known about similarities between these two diseases concerning the distribution of the γδTCR lymphocytes in peripheral blood and BAL and their association to various cell surface molecules. Therefore, the present study was undertaken to determine and compare the distribution of T-cells expressing γδTCR in blood and BAL of patients with sarcoidosis, HP and idiopathic pulmonary fibrosis (IPF) and healthy controls, applying the method of immunophenotyping by flow cytometry. Furthermore, T-cells of the above-mentioned patient groups were analysed to correlate the different T-cell subsets with the γδTCR expression.

Materials and methods

Study population

All patients were diagnosed on the basis of conventional clinical criteria and results of transbronchial lung biopsy.

Sarcoidosis. The group of sarcoidosis patients included 31 individuals, 11 males and 20 females, aged 20–55 yrs. Six of them were current smokers and seven were ex-smokers. Eleven sarcoidosis patients had enlarged hilar and/or mediastinal lymph nodes (stage I), and 20 combined enlarged thoracic lymph nodes and parenchymal lung infiltrates (stage II). Bronchoalveolar lavage was performed in 20 of the 31 patients.

Hypersensitivity pneumonitis (HP). Thirteen individuals with HP were examined, 3 females and 10 males, aged 31–65 yrs. Two were smokers and three were ex-smokers. Diagnoses were based on typical case histories, the presence of antigen-specific antibodies, an abnormal chest X-ray and/or a restrictive ventilation pattern [12, 13]. This group, including patients with farmers' lung disease (n=6), isocyanate alveolitis (n=1), and bird/pigeon fanciers' lung (n=6), was characterized by the typical symptoms mentioned above and in BAL by a CD4/CD8 ratio below 1.0, increased numbers of activated T-cells (CD3/HLA-DR, CD25) and natural killer cells (CD57+/CD56+). In five of the patients, BAL was performed 20 h after antigen provocation, and showed neutrophils compatible with an acute form of HP.

Idiopathic pulmonary fibrosis (IPF). Twelve individuals with IPF, 10 males and 2 females, aged 43–78 yrs (mean age 57±14 yrs) were investigated. Four were smokers and one was an ex-smoker. There were no clinical signs of HP and no histories of occupational or private exposure to organic or inorganic dusts in this group. The patients were without evidence of collagen vascular disease. In this group, BAL lymphocytes were not elevated, and the CD4/CD8 ratio in the BAL was within the normal range (1.1–3.5). A characteristic feature of BAL was an increased percentage of granulocytes (neutrophils and/or eosinophils) up to 15%.

Control group. The control group consisted of 21 healthy volunteers, 12 males and 9 females, aged 22–56 yrs. Seven were smokers and two were ex-smokers. This group was only investigated for blood studies; a BAL was not performed.

BAL procedure and BAL cell preparation

BAL was performed using 100 ml sterile saline solution. The percentage return of lavage fluid was determined for each BAL, and ranged between 48–85 ml (60±11 ml, n=32; mean±SD). The lavage fluid was filtered through a single layer of coarse gauze and centrifuged (200g, 10 min 4°C) to sediment cellular material for cytological and flow cytometric examinations. A trypan blue exclusion test for cell viability was performed, indicating that more than 95% of cells were viable. The number of cells presented in BAL was determined, and an aliquot of the cell suspension was used to prepare cytospin slides (CytoSpin 3, Shandon, Frankfurt, Germany). The slides were stained using May–Grünewald/Giemsa, and a differential cell count was performed by examination of 300 nonepithelial cells.
Monoclonal antibodies (MoAbs)

The following conjugated MoAbs were used to detect lymphocytes, T-cells and T-cell subpopulation: fluorescein isothiocyanate (FITC)-conjugated anti-CD45 (pan leucocyte antigen), phycoerythrin (PE)-conjugated anti-CD8 (leu-2a, recognizing CD8+ "cytotoxic/suppressor" T-cells), PE-conjugated anti-CD4 (leu-3a, recognizing CD4+ "helper/inducer" T-lymphocytes), PE-conjugated anti-CD3 (leu-4, recognizing all CD3+ T-lymphocytes), PE-/or FITC-conjugated anti-CD25 (recognizing the interleukin-2 (IL-2) receptor), FITC-anti-CD4/PE-anti-CD8 (leu-3a/leu-2a) and FITC-anti-CD45/PE-anti-CD14 (monocyte specific). To determine the TCR phenotype of T-lymphocytes, anti-TCR $\alpha/\beta$-1 (identifying all $\alpha/\beta$ T-lymphocytes) and anti-TCR $\gamma/\delta$ (recognizing all $\gamma/\delta$ T-lymphocytes) were used in the fluorescein (FITC)-conjugated form. Control antibodies included isotype-matched PE-conjugated and FITC-conjugated mouse antibodies. The MoAbs were purchased from Becton-Dickinson, Heidelberg, Germany.

Immunofluorescent assays

Ten microlitres of each of the fluorescent MoAbs were added to 50 µl heparinized blood. The mixture of blood and MoAbs was kept at room temperature in the dark for 15 min. Then the erythrocytes were lysed with 2ml of erythrocyte lysis buffer (Becton Dickinson) for 15 min. After centrifugation at 200xg for 5 min, the pellet was washed twice with phosphate-buffered saline (PBS). Finally, the cells in the washed pellet were resuspended in 1% paraformaldehyde, and kept at 4°C until flow cytometric analysis.

BAL cells (2×10^5, 50 µl) were incubated for 30 min at 4°C in the darkness, with 10 µl of the indicated FITC- and PE-MoAbs. After incubation, the cells were washed twice with PBS. The staining of the immunofluorescent-labelled cells was resuspended in 300 µl of 1% paraformaldehyde in PBS. To distinguish between damaged and intact cells in fixed flow cytometric samples, the vital nuclei acid stain LDS 751 (Exciton, Dayton OH, USA) [14] was used.

![Flow cytometric analysis of bronchoalveolar lavage (BAL) lymphocytes in a representative sarcoidosis patient. Lymphocytes (R1) of the BAL were distinguished from macrophages and granulocytes by FITC-conjugated anti-CD45 (Fl-1) and side scatter plots (SSC) (Panels a and b). Intact lymphocytes (R2) were discriminated from damaged cells by forward light scatter (FSC) and LDS 751 stain (Fl-3) (Panel c). By a mixture of FITC-anti-CD45 (Fl-1) and PE-anti-CD14 (Fl-2), the purity of the lymphocyte gate (CD45+/side scatter) was controlled (Panel d). Panel d indicated that most of the cells (>97%) in this gate were lymphocytes, because they were CD45-positive and CD14-negative. FITC: fluorescein isothiocyanate; PE: phycoerythrin.](image-url)
Flow cytometric analysis

A fluorescence-activated cell sorter system (FACS-Star-Plus, Becton Dickinson) with an argon laser (488 nm, air cooled) was used. Acquisition and analysis were carried out using the Lysis 1.0 programme. For each test, at least 10,000 cells were acquired.

Blood lymphocytes were discriminated from blood monocytes or neutrophils by characteristic forward light scatter (FSC) and right side light scatter (SSC) profiles. The characteristic scatter conditions were controlled by staining with a mixture of FITC-conjugated anti-CD45 and PE-conjugated anti-CD14 (leucogate). For two-colour immunofluorescence studies, the proportions of lymphocytes with positive staining for each antibody were calculated by setting four quadrants. Based on control samples, we divided the dot plots for unstained cells (lower left), and single stained cells: FITC-stained cells (lower right), PE-stained cells (upper left), and double-stained cells (upper right). Unstained cells were required to assess cellular autofluorescence. Directly conjugated subclass control cells (labelled with immunoglobulin G1 (IgG1)-FITC and (IgG2)-PE) will indicate nonspecific binding in direct staining experiments. Controls were subtracted, and the results were expressed as percentage of analysed gated cells.

BAL lymphocytes were distinguished from macrophages and granulocytes by fluorescence with FITC-conjugated anti-CD45 and side scatter plots (SSC) (fig. 1). However, most of the cells (>97%) in this fluorescence/side scatter gate are lymphocytes; this gate may also include small numbers of nonlymphoid cells (e.g. CD14-positive monocytes). To evaluate the number of these cells, they were analysed with an antibody mixture of FITC-conjugated anti-CD45 and PE-conjugated-anti CD14. Gated lymphocytes (by CD45+/side scatter gate) were FITC anti-CD45 positive, and negative by PE anti-CD14 fluorescence (fig. 1d).

To detect T-cell subsets in the BAL, a mixture of FITC anti-CD45 and specific antibodies for the T-cell subsets conjugated with PE was used. For the identification of TCR bearing lymphocytes, fluorescence PE-conjugated anti-CD3, anti-CD4 or anti-CD8, and FITC-conjugated TCRαβ or FITC-conjugated anti-TCRγδ were used.

Statistical methods

Quantitative data were expressed as the mean±standard deviation (sd). Wherever relevant, the Kruskal-Wallis test with a Monte Carlo estimation (by 100,000 tables) of p-values was used to compare the four and three groups of patients, respectively. To compare the data location of blood γδT-lymphocytes between two patient groups, e.g. control and sarcoidosis, control and HP, control and IPF, or sarcoidosis and HP, the exact Wilcoxon rank sum test was used. Two-sided test p-values were calculated, and p-values of <0.05 were considered significant. Calculations were performed on a personal computer using StatXact statistical package (Cytel-Software, Corp., Cambridge, MA, USA).

Results

Distribution of blood CD3+ γδTCR cells

Using two-colour direct immunofluorescence with the anti-γδTCR and anti-CD3 MoAbs, flow cytometric analysis demonstrated that patients with pulmonary sarcoidosis and HP show a significant increase in the proportions of CD3+γδ+ cells compared with normal subjects (fig. 2). Normal individuals had 3.2±1.4% (range 0.2–6.6%) of blood T-cells that were CD3+ TCRγδ+, and sarcoidosis patients had 8.3±7.9% (range 0.8–29.4%). Using 7.4% (mean of the normal control group plus 3×sd) as the upper level of normals, a subgroup of 15 of the 31 (48%) individuals with sarcoidosis had CD3+γδTCR+ lymphocytes above this level. Patients with HP had 5.8±3.5% (range 1.3–11.5) of CD3+γδTCR lymphocytes in their blood, whereas IPF patients had 3.2±1.3% (range 0.9–7.4) (fig. 2). In the former group, five of the 13 subjects (33%) expressed γδTCR on more than 7.4% of their T-cells. There was no significant difference between HP and sarcoidosis patients in the proportion of CD3+γδTCR+blood T-cells.

To determine the absolute number of CD3+γδ+ cells in the blood, data from the total white blood cell counts, cell differentials and the proportions of CD3+γδ+ cells measured by cytofluorometry were calculated. In sarcoidosis patients, 187.5±112.9 cells·µl−1 were CD3+γδ+ TCR (mean±sd), whereas in normal subjects CD3+γδ+ cells were present in low numbers (77.7±18.2 cells·µl−1) (sarcoidosis versus control p<0.02). In HP patients, 189.2±65.4 cells·µl−1 were CD3+γδTCR (HP versus control p<0.01). In patients with IPF only 75.7±49.7 cells·µl−1 had the γδTCR. These data indicate an increase in the total number of circulating blood CD3+γδ+ cells in individuals with pulmonary sarcoidosis and HP.
Table 1 – Comparison of patients suffering from sarcoidosis with higher and lower levels of γδTCR-lymphocytes in blood

<table>
<thead>
<tr>
<th></th>
<th>Patients with higher CD3+ γδTCR level</th>
<th>Patients with lower CD3+ γδTCR level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(≥7.4%) n=15</td>
<td>(&lt;7.4%) n=16</td>
</tr>
<tr>
<td>Female/Male</td>
<td>9/6</td>
<td>60/40</td>
</tr>
<tr>
<td>Age yrs</td>
<td>36±8.8</td>
<td>34±8.9</td>
</tr>
<tr>
<td>Stage I</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Stage II</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>Smokers</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>Exsmokers</td>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Blood CD4/CD8 ratio</td>
<td>0.74±0.36</td>
<td>1.17±0.59</td>
</tr>
</tbody>
</table>

*: mean±sd. #: p<0.05. TCR: T-cell receptor.

Table 1 compares the data of sarcoidosis patients with increased (≥7.4%) and normal proportions (<7.4%) of blood CD3+ γδTCR cells. With respect to age, sex and smoking behaviour, patients with increased numbers of CD3+ γδTCR cells did not differ significantly from those with normal numbers of these T-cell subpopulations in the blood. Comparison of the blood CD4/CD8 ratios in these two groups demonstrated a significantly lower CD4/CD8 ratio in the group of patients with higher CD3+ γδTCR levels (p<0.05). If the CD4/CD8 ratios were compared with the ratio of healthy controls (1.49±0.52, n=21), a significant difference existed only between sarcoidosis patients with higher CD3+ γδTCR levels and the control group (p<0.01). The CD4/CD8 ratios of patients suffering from HP, both with normal and elevated CD3+ γδTCR levels in blood, and from IPF were in the normal range (1.20±0.94 and 1.25±1.09 respectively).

Correlation between CD4+ and CD3+ γδTCR lymphocytes in blood

According to the differences between the CD4/CD8 ratio of control individuals and sarcoidosis patients, we compared the percentage of CD4+ lymphocytes in blood of healthy subjects with that of sarcoidosis patients. The data indicated a significant decrease of CD4+ lymphocytes in the blood of sarcoidosis patients (n=31) versus normal volunteers (n=21) (26±10.9 versus 42±6.6; p<0.01) (fig. 3). (13 out of 15) sarcoidosis patients who expressed γδTCR on more than 7.4% of their lymphocytes had less than 30% of CD4+ lymphocytes (impoverishment of CD4 in blood). In all normal individuals examined, the percentage of CD4+ blood lymphocytes was higher than 30%.

The percentages of CD4+ lymphocytes in patients with IPF and HP did not differ from normal values (36±11.0% in IPF and 37±11.8% in HP). Only two HP patients expressing γδTCR on more than 7.4% of their lymphocytes had a CD4+ percentage below 30%.

Association between γδTCR of blood T-lymphocytes and accessory molecules

Association between γδTCR of blood T-lymphocytes and the accessory molecules CD4 or CD8 was performed in blood of sarcoidosis and in healthy controls. The data on seven patients indicated that 46±13.9% of γδTCR+ T-cells were CD8-positive, and 50±15.1% of the γδ+ cells were neither CD8 nor CD4, having a "double negative" phenotype. The number of cells scored as γδ+ CD4+ (3.5±2.8%) was equivalent to the background levels of staining, indicating that γδTCR lymphocytes were CD4-negative. The association between the γδTCR expression and the phenotypes CD4+CD8- or CD4-CD8+ were irrespective of whether the sarcoidosis patients had elevated or normal percentages of circulating γδ+TCR lymphocytes. Concerning this association, no significant difference existed between sarcoidosis patients and healthy controls; in the last-mentioned group 38±4.1% of γδTCR- cells also bear CD8, and 62±3.1% had a "double-negative" phenotype (CD4-CD8-).

To determine whether γδTCR+ lymphocytes had been recently activated, the expression of the IL-2 receptor (CD25) was evaluated. Double staining experiments with PE-conjugated anti-CD25 and FITC-anti-γδTCR from all tested individuals (eight sarcoidosis patients and four healthy controls) demonstrated that the γδTCR+ lymphocytes are CD25-negative (0.4±0.5%, n=12).

Distribution of CD3+ γδ+T-cells in BAL

The evaluation of lymphocytes present in the BAL indicated a higher percentage of CD3+ γδTCR+ cells in HP patients than in IPF, p<0.05 (fig. 4, table 2). The percentage of CD3+ γδTCR+ of patients with sarcoidosis was not significantly different from that of patients with HP or IPF.
In contrast to percentage values, the total amounts of CD3+ γ/δTCR+ (table 2) showed significant differences in sarcoidosis, HP and IPF (sarcoidosis versus IPF, p<0.02; HP versus IPF, p<0.05 and sarcoidosis versus HP, p<0.05).

The 20 sarcoidosis patients had 4.2±3.6% (range 0.8–12.8%) of CD3+ γ/δTCR+ cells. Ten of these sarcoidosis patients belonged to the subgroup with increased blood levels of CD3+ γ/δTCR+ (≥7.4% of CD3+ γ/δTCR+ cells). In addition, the cellular distribution of the BAL fluids of the two sarcoidosis subgroups, with increased and normal blood levels of CD3+ γ/δTCR+, was compared (table 3). Sarcoidosis patients with higher blood CD3+ γ/δTCR+ levels showed significantly decreased total cell numbers·ml⁻¹ BAL and lymphocytes·ml⁻¹ (p<0.05), compared with the other sarcoidosis subgroup. Corresponding with the higher percentage of blood CD3+ γ/δTCR+ levels, this subgroup also had an increased percentage of CD3+ γ/δ+ in the BAL (p<0.05), but the total number of CD3+ γ/δTCR+ cells·ml⁻¹ was not different in the two patient groups. Calculating the BAL CD4/CD8 ratios of the two groups, no significant difference was observed.

Comparison of BAL data obtained from HP patients with increased (5 out of 13 patients) and normal (8 out of 13) blood CD3+ γ/δ+ levels demonstrated no significant difference with respect to BAL CD3+ γ/δ+ levels (7.7±8.2 (n=5) versus 9.3±9.07 (n=8); p>0.05) and CD4/CD8 ratios (0.78±0.54 (n=5) versus 0.63±0.31 (n=8); p>0.05).

Compared to IPF in the BAL of patients suffering from sarcoidosis and HP the absolute number and the percentage values of lymphocytes were significantly higher.
**Discussion**

Our data demonstrate elevated levels of T-cells bearing the CD3+ γδ TCR in blood of patients suffering from sarcoidosis and HP. In both patient groups, this expansion of γδ T-cells was clearly defined and significantly different from blood of controls and patients suffering from IPF, when considering both the mean value of the percentage or the total numbers of CD3+ γδ TCR+ cells. However, the proportions of γδ TCR+ T-lymphocytes in blood of sarcoidosis and HP patients were quite variable, and ranged from 1.0–29%, and more than 50% of individuals in both patient groups had circulating γδ TCR+ T-lymphocytes in the normal range.

Our data on γδ TCR expression in sarcoidosis patients are in agreement with those of BAlbi et al. [6], who found increased numbers of αβ T-cells in the blood of a subgroup of individuals with sarcoidosis. Our study confirmed that CD3+ αβ T-cells were actually CD3+ γδ T-cells. In contrast to these data and ours, Tazi et al. [15] did not find an increase of the absolute number of CD3+ γδ T-cells in patients with sarcoidosis. They suggested that an increased proportion of circulating CD3+ γδ T-cells resulted from a reduction in the number of lymphocytes bearing the αβ TCR. Our results clearly demonstrate that the increase in γδ T-cells was due to an increase in the absolute number of blood γδ T-cells, but it must be considered that this phenomenon may differ in various study populations of sarcoidosis. Furthermore, Tazi et al. [15] demonstrated that the γδ T-cells in the blood play a limited role in the ongoing immune response in established tuberculosis. These results are strikingly different compared to the important increase in number of γδ T-lymphocytes observed in the lung and lymph nodes of mice exposed to Mycobacterium tuberculosis [16]. In addition, Forrester et al. [17] described that a subgroup of sarcoidosis patient, 6 out of 11, (54%) had increased γδ T-cells in blood, whereas in patients with chronic beryllium disease only 2 out of 9 individuals (22%) had elevated blood γδ T-cells.

To understand why certain patients suffering from sarcoidosis have increased numbers of γδ T-cells, we compared some personal and clinical data of the patient groups with higher and normal levels of γδ TCR. Concerning age, sex, smoking behaviour and sarcoidosis stage, no significant difference existed between the two groups. However, a significantly lower CD4/CD8 ratio was demonstrated in the group of patients with higher γδ TCR levels. In addition, our data clearly demonstrate a good correlation between the decrease of CD4+ blood cells and the amplified appearance of γδ TCR expression in patients with sarcoidosis. It seemed possible, that in sarcoidosis the expansion of blood γδ T-cells was due to an increase of CD8, corresponding to the decrease of CD4 cells. In contrast, in HP patients the specific expansion of γδ T-cells seems to be independently regulated, irrespectively of a CD4 decrease. The clear correlation between CD4+ decrease and γδ TCR+ increase may be of particular interest in sarcoidosis patients.

Moreover, our study indicates that the γδ TCR of blood T-lymphocytes was associated either with CD8 in approximately 50% or with a "double negative phenotype" (CD4−CD8−) on the remaining 50% of the γδ T-cells. Similar to our results, which demonstrate that only 3.5±2.8% of the γδ T-cells bear CD4, Inghirami et al. [18] reported that all γδ TCR bearing peripheral lymphocytes lack detectable CD4, whilst approximately 60% of the γδ TCR thymocytes express low density CD4. Although CD4 expression on peripheral blood γδ T-cells is even less common than CD8 expression, CD4 γδ T-cells had been identified and cloned [19]. An further study [20] showed that approximately 30% of the γδ T-cells in the blood were CD8+, and Bucy et al. [20] and Inghirami et al. [18] concluded that γδ T-cells are resting, mature T-cells that probably play a primary role in suppressor/cytotoxic phenomena and recognize antigens in association with classical or non-classical class I MHC molecules. Because all thymic γδ T-cells are CD8+, it will be important in this context to determine whether CD8 expression by γδ T-cells represents a normal stage of maturation, or occurs only after cell activation.

Finally, our data indicate, irrespective of elevated or normal levels of circulating γδ T-cells, that these cells were IL-2 receptor-negative (CD25−γδ TCR+), suggesting a lack of activation. Interestingly, looking for the expression of other cell surface antigens, e.g. CD45RO as a marker of memory cells, it seemed that γδ T-cells have been preactivated in vivo [21]. It has to be considered that certain activation signals on αβ T-cells may have opposite effects on γδ T-cells [22], and that an activation of γδ T-cells does not correspond to the expression of usual activation markers, such as CD25.

In addition, our data demonstrate an increase of γδ TCR+ cells (absolute numbers) in BAL of patients suffering from HP and sarcoidosis, compared to BAL of IPF patients. In the subgroup of sarcoidosis patients with higher percentages of CD3+ γδ TCR+ level, an increased percentage of CD3+ γδ TCR+ cells in BAL was also observed. These data suggest that the γδ T-cells were not restricted to one compartment, because a corresponding increase in blood and lung (BAL) was seen in sarcoidosis patients.

In agreement with our results, Trentin et al. [23] found that the absolute number of γδ TCR-bearing T-cells was increased in the BAL of patients suffering from farmers’ lung disease. Our study also demonstrated an increase of CD3+ γδ T-cells in the blood and BAL of patients with HP caused by other antigens. In contrast to sarcoidosis patients, the comparison between blood and BAL CD3+ γδ TCR+ levels of HP patients does not show a clear correlation.

Our study suggests that this CD3+ γδ TCR-lymphocyte subpopulation might be of pathogenetic relevance in the blood and lung of patients with sarcoidosis and HP. These are two different interstitial lung disorders, with specific pathogenetic mechanisms and characteristics. Semenzato [24] concluded that the mechanisms accounting for alveolitis in sarcoidosis are an in situ cellular proliferation and a cellular Redistribution from the peripheral blood to the site of the disease activity (i.e. the lung). Redistributed cells are especially the CD4+ helper related cells. In patients with HP, the BALs are characterized by...
cells bearing suppressor/cytotoxic phenotypes. The expansion of cells with the mentioned characteristics in the BAL of these patients is likely to be related to a local immune response to the antigenic stimulus. It has also to be considered that, in contrast to sarcoidosis, the BAL of patients with HP contains CD8+ cells as the dominant lymphocyte subpopulation. However, the absolute number of γδ+ T-cells in BAL from HP patients is not higher than in BAL from sarcoidosis patients.

In conclusion, our data demonstrate elevated levels of γδ+ T-cells in sarcoidosis and HP patients suggesting a pathogenetic role of γδTCR-bearing T-cells. It has not yet been clarified whether the bias in CD3+ γδTCR expression is a causal factor for the development of such a disease, or whether it is a consequence of it.

Acknowledgements: The authors would like to thank G. Borowiński, C. Kreckel and A. Urbanski for their technical assistance and P. Degens for statistical assistance.

References