Sarcoidosis: immunopathogenetic concepts and their clinical application

J. Müller-Quernheim

**ABSTRACT:** Our understanding of the immunopathogenesis of sarcoidosis has been advanced by studies of bronchoalveolar lavage cells. Activated macrophages and T-cells have been identified in different compartments of the sarcoid lung and the characteristics of the activation suggest that the cells become activated in the course of a normal immune response. The immune cells communicate via a cytokine network and the measuring of cytokine levels yields subgroups of sarcoidosis patients with different courses of the disease indicating different states of activation of the disease-mediating immune cells. The causative agent of sarcoidosis has not yet been identified; however, some of the described mechanisms can be clinically applied either to detect patients at risk of deterioration or to develop new therapeutic strategies. Using these approaches methotrexate, pentoxifylline and thalidomide have been identified as drugs which effectively suppress sarcoid inflammation and the serum level of soluble interleukin-2 receptors has been delineated to be a serum marker of sarcoid inflammation. Furthermore, analysing the pulmonary cytokine network in sarcoidosis will yield new staging parameters possibly supplying prognostic information and guiding therapeutic intervention.

**Epidemiology**

Since many individuals with sarcoidosis are asymptomatic, estimates of the incidence rates and prevalence figures depend mainly on the way in which epidemiological data are generated. In Europe, sarcoidosis is the most frequently observed interstitial lung disease of unknown aetiology. The prevalence rates range from 64 patients per 100,000 population in Sweden to 9 per 100,000 population in Italy with intermediate numbers observed in Denmark (53 per 100,000), Germany (43), Ireland (40), Norway (27), The Netherlands (22), the UK (20), Switzerland (16) and France (10). The prevalence for the Caucasian population of North America is 3 and for Afro-Americans 47 per 100,000. Sarcoidosis is found in all races, affecting slightly more females than males. Most commonly, it manifests in adults aged 20–45 yrs, although all ages can be affected.

Differences in the pattern of organ involvement and the severity of the disease have been observed according to race and ethnic background. Erythema nodosum associated with acute disease and good prognosis is most frequently seen in young Caucasians, as originally described by Löfgren [6]. Lupus pernio and other cutaneous manifestations of sarcoidosis, considered to be stigmata of chronic disease [7], appear more frequently in African-Americans than in Caucasians [8].

Only rough estimates of the mortality rates of untreated sarcoidosis are available. If untreated it is associated with a mortality rate of ~5%. In an epidemiological study from Denmark with a median follow-up of 27 yrs, an excess...
mortality from sarcoidosis and sarcoidosis-related diseases was perceived in the first 20 yrs in patients with advanced radiological findings and deteriorated lung function. Although the mortality of the sarcoid cohort was higher than that of the general population the difference was not statistically significant [9, 10]. This number may differ in other ethnic groups [11, 12] or cohorts with increased frequencies of certain manifestations, such as cutaneous sarcoidosis [13, 14].

Genetics

Numerous reports on familial sarcoidosis, human leukocyte antigen (HLA) linkages and divergent prevalence rates and clinical appearances in different races indicate the existence of genes predisposing for sarcoidosis [8, 15–18].

Recently, maps of markers that cover the entire murine and human genomes have enabled the chromosome localization of many genes that predispose to autoimmunity in humans and animal models (reviewed in [19]). Using highly polymorphic markers in a family study of Crohn’s disease a susceptibility locus was mapped to a region on chromosome 16 [20]. For sarcoidosis these studies are still in their infancy [21]. Many autoimmune or immune-related diseases cluster in families and the degree of familial clustering of diseases can be estimated from the ratio between the risk of siblings suffering from a given disease and the population prevalence. If the sibling’s risk is equal to the general prevalence familial clustering is not observed and the ratio (λs) is close to 1.0. When the siblings are at a greater risk of developing a given disease λs exceeds 1.0. For the diseases shown in table 1, λs is greatly elevated. This is true for nongranulomatous diseases and granulomatous diseases such as Crohn’s disease and primary biliary cirrhosis, which share some immunological features with sarcoidosis. The clustering of diseases in families is caused by genetic factors, environmental factors, or both. The λs for the major histocompatibility complex (MHC) is available for some diseases. If the overall λs equaled the MHC λs this would imply that only one MHC gene accounts for the clustering. It is evident from table 1 that in all diseases where data are available λs greatly exceeds MHC λs, implying that the linkage of the MHC region cannot entirely account for the clustering. In Crohn’s disease (λs=20, MHC λs=1.3) genome scans have identified a susceptibility locus outside the MHC on chromosome 16 [20]. Interestingly, the gene of Blau’s syndrome (familial granulomatosis), an autosomal, dominantly Mendelian-inherited disease characterized by multiorgan inflammation with granulomatous arthritis, skin rash and uveitis, has been mapped to the same region [27].

Estimating λs of sarcoidosis, from the numbers available for Germany [16, 28], yields a range from 8 to 20.0 and points very strongly to the existence of predisposing genes (table 1). For berylliosis, an occupational disease clinically indistinguishable from sarcoidosis, an association between this disease and the presence of a glutamine residue at position 69 (Glu69+) of the β1-chain of the HLA-DP molecule has been identified [29]. A study analysing this locus in a series of 24 patients with sarcoidosis failed to demonstrate an association [30] but two others [31, 32] examining 41 and 38 patients, respectively, succeeded in showing an association. The author’s group analysed 17 familial clusters for the presence of Glu69+ and found an over-representation; however, this allele was not shared by any of three sib triplets included in this study [33]. These discordant findings of over-representation of distinct HLA genes in sarcoidosis patients and the lack of linkage in sarcoidosis families might be caused by a more complex genetic background, as indicated by ethnic differences in the frequency of the genes in question [31]. Heterogeneity of the disorder itself and polygenic triggering of its course might also be involved.

On chromosome 17 another gene polymorphism, a 300 base-pair (bp) deletion/insertion polymorphism in exon 16 of the angiotensin-converting enzyme (ACE) gene, is thought to be associated with sarcoidosis [34–36]. In a Japanese study an excess of genotypes ID or DD was observed in female patients [34], but in another investigation from Japan there were no significant differences in the I/D ratio and the genotype distribution between the patients with sarcoidosis and controls [37]. The testing of different models of inheritance in our deoxyribonucleic acid (DNA) bank of familial sarcoidosis clusters revealed no cosegregation of the ACE gene with familial sarcoidosis [38], giving further support to the notion that the ACE polymorphism is not responsible for the development of sarcoidosis [37]. However, an over-representation of the allele with the insertion was observed in both family members with and without sarcoidosis, which may indicate a participation of this allele in a multifactorial disease process. The I/D polymorphism does not alter the ACE protein structure.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Population frequency %</th>
<th>Sibling risk %</th>
<th>λs</th>
<th>MHC λs</th>
<th>Reference</th>
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<td>17</td>
<td>6</td>
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<td>8</td>
<td>8.3</td>
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<td>15</td>
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<tr>
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<td>8–20</td>
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λs: ratio between the risk of siblings suffering from a given disease and the population prevalence; MHC: major histocompatibility complex; SLE: systemic lupus erythematosus. (Modified from [19].)
but it influences the serum ACE level. Compared with heterozygote individuals, the DD genotype is associated with higher and the II genotype with lower ACE levels. ACE serum levels are clinically employed to gauge the granuloma burden of the body [39] and in the future the use of genotype-corrected normal ranges will improve the low sensitivity of this marker [36, 40].

Aetiology

Many agents have been implicated as possibly providing the stimuli that elicit the granulomatous response of sarcoidosis. As early as 1905, C. Boeck described sarcoidosis as "a bacillary infectious disease, which is either identical to tuberculosis or closely related to it"; however, proof of this hypothesis remains elusive and there is an ongoing discussion about this question. Besides mycobacteria and inorganic agents, even viruses, e.g. herpesviruses, seem to be capable of inducing sarcoid-type granulomas [41].

High titres of antibodies against lymphotropic DNA viruses (Epstein-Barr virus (EBV), human herpesvirus (HHV), cytomegalovirus), parainfluenza, rubella and mycobactériophages have been found in patients with sarcoidosis. Using the polymerase chain reaction (PCR) viral DNA can be identified in tissues from varying numbers of sarcoidosis patients. Adenovirus DNA was found in a small percentage of patients with sarcoidosis [42] and HHV-8 DNA sequences were detected in significantly higher proportions of sarcoid than of nonsarcoid tissue from lung, lymph nodes, skin and oral tissues [43]. Whether these associations are causal or not and whether they point to a general association between viruses and granuloma require further studies. HHV-8 DNA is found in Kaposi's sarcoma and in the blood and other body fluids of groups at high risk of Kaposi's sarcoma [44, 45]. Studies using better techniques to seek viral sequences in blood [46], tissues [47] or body fluids other than blood [48] and studies identifying serum antibodies to HHV-specific peptides [49, 50] indicate that HHV-8 infection in populations not at risk of Kaposi's sarcoma may be more prevalent than previously thought. In line with this notion is the observation of Di Alberti et al. [51], who identified different HHV-8 sequences from different body sites of persons infected with human immunodeficiency virus, which suggests multiple infections with HHV in these patients that are possibly associated with disease processes. However, at present a viral cause of sarcoidosis has not been substantiated by viral cultures or unequivocal tissue analysis.

Using methods of histology, bacterial cultures and molecular biology the presence of acid-fast rods, mycobacterial DNA and mycobacterial ribonucleic acid (RNA) has been demonstrated in sarcoidosis [51–56]. However, other investigators have not been able to reproduce these findings, and based on the assumption that in a range of 2,500–10^6 host cells of established lesions more than six to 15 organisms are required to play a pathogenic role, it has been concluded that Mycobacterium tuberculosis or other mycobacteria are not involved in sarcoidosis [57–59]. An interesting point is the detection of 16S ribosomal RNA (rRNA) sequences of as yet unidentified mycobacteria in sarcoid lymphoid and skin tissue by Di Alberti et al. [43] who could not find DNA sequences of M. tuberculosis in these samples. Virekura et al. [60] used a sequence capture PCR, which is about 100-fold more sensitive than conventional PCR protocols, to seek the mycobacterial insertion segment 6110 and the mycobacterial DR region. Although in this study M. tuberculosis was detected in samples of paucibacillary tuberculosis, this approach did not obtain positive results for sarcoidosis, which supports previous studies suggesting that M. tuberculosis does not play a pathogenic role in sarcoidosis in most patients. Owing to the sequences of the chosen primers Virekura et al. [60] could not detect the sequences reported by Di Alberti et al. [43]. Thus, these studies support each other in the conclusion that M. tuberculosis cannot be found in sarcoid tissues. The relevance of the yet undefined mycobacteria needs to be determined.

Epidemiological data and similarities with other infectious diseases support the hypothesis that sarcoidosis is induced by an infectious organism. Seasonal clusterings of sarcoidosis in the months of June and July [61], time and space clusters [62, 63], an increased incidence in health workers [64] and the transmission of sarcoidosis by transplant [65] have been observed and further support the hypothesis of transmissible sarcoid-inducing agents. For example, Borrelia burgdorferi has recently been proposed as a possible cause of sarcoidosis [66, 67], although data obtained by the author's group do not support this hypothesis [68]. Furthermore, a recent report has demonstrated the presence of antibodies against Chlamydia pneumoniae in 20 out of 24 sera from patients with sarcoidosis [69]. However, in other study populations DNA of C. pneumoniae could not be detected in tissue samples of sarcoid lung [70], and patients with sarcoidosis did not exert an increased seroprevalence of antibodies against Chlamydia spp. [63]. In most countries, including Germany, there is a high anti-Chlamydia seroprevalence and preliminary results from the author's laboratory do not show an elevated anti-Chlamydia seroprevalence in sarcoidosis. Therefore, the presence of Chlamydia spp. in patients with sarcoidosis should be evaluated with scrutiny. The presence of virus- and bacteria-specific antibodies in high titres might reflect generalized B-cell activation in sarcoidosis and does not necessarily indicate a causal relationship.

In this context an epidemiological study from Denmark is of interest, which raises a dual infection hypothesis in combination with a genetic susceptibility hypothesis for multiple sclerosis. Infection with a hypothetical, widespread "multiple sclerosis retrovirus" is a prerequisite for the development of multiple sclerosis, but the disease develops only in individuals who were infected with EBV around puberty or later in life and are genetically susceptible [71]. Owing to its clinical heterogeneity such a hypothesis is difficult to test for sarcoidosis.

Most interestingly, there are case reports of sarcoidosis patients who suffered from a relapse of sarcoidosis in a transplanted lung despite receiving immunosuppressive therapy [72–74]. Conversely, in two out of four cases patients receiving a lung or other organs from a donor who had a spontaneous remission of sarcoidosis in the past were observed to develop sarcoid-like lesions without suffering from sarcoidosis [73]. Others report the transmission of sarcoidosis by cardiac transplant [65]. These observations suggest that the aetiological agent hides within the lung and/or other compartments of the body.

In view of the studies described above and the fact that only a few years
staining of sarcoid tissue. CHASE [79] proposed a standardized procedure for the preparation of the test reagent still in use today. SELTZER [80] performed comprehensive studies establishing the practicability of the test as a diagnostic tool and the Kveim-Siltzbach test is still applied in a number of countries for the diagnosis of sarcoidosis. So far, the nature of the Kveim reagent remains obscure. Denaturing experiments have revealed that the active, granuloma-inducing principle should consist of a protein and depends on its three-dimensional structure [81].

T-cells from sarcoid patients do not proliferate after stimulation with Kveim reagent [82] and in spite of many attempts it has not been possible to develop an in vitro test for clinical routine purposes [83]. Employing different immuno-electrophoretic methods, REUTER et al. [84] demonstrated 3–11 precipitation lines in the Kveim reagent. Immunohistochemical stainings with antisera or monoclonal antibodies against Kveim reagent showed reactivity to epithelial cells in sarcoid granulomas and, to a lesser extent, in tuberculoid granulomas [84, 85]. Additional studies revealed that an anti-Kveim reagent monoclonal antibody recognizes both epithelial cells and giant cells from granulomas from patients with summer-type hypersensitivity pneumonitis, Crohn's disease, foreign body granuloma and Wegener's granulomatosis. These data show that at least one fraction of this preparation consists of proteins related to granuloma development in general and are not specific to sarcoidosis.

In an ongoing study in the author's laboratory the bacterial or mycobacterial DNA was searched for in the Kveim reagent. DNA coding for the 16S rRNA, which is an essential part of the bacterial ribosome, could not be detected in Kveim reagent or a sarcoid spleen. Thus, the negative results obtained do not support the hypothesis that the cutaneous granuloma at the site of a positive Kveim reaction are caused by bacteria [86]. However, the possibility that bacterial products such as superantigens or membrane fragments [87] induce these reactions cannot be excluded. Numerous immunobiological studies, especially those dealing with lymphocytes, have shown that most of the immune characteristics of the sarcoid reaction are shared with the normal immune response, supporting the hypothesis of an infectious aetiology of sarcoidosis [88, 89]. The fact that bacterial DNA cannot consistently be found in Kveim reagent or in sarcoid lesions suggests that, if an infectious aetiology of sarcoidosis exists at all, bacteria or viruses may trigger the sarcoid response, which may be maintained by undegradable products of these species or by autoaggressive or cross-reacting host mechanisms after the causative agent has been cleared, as has been demonstrated for infectious arthritis [87]. This notion is further supported by studies indicating that the active principle of the Kveim reagent is of a proteinous nature [81, 90].

Exposure to inorganic or organic dusts can cause diseases that are clinically and histologically indistinguishable from sarcoidosis. The most prominent example is berylliosis, for which a genetically defined susceptibility has been identified. Those individuals with a glutamate instead of a lysine in position 69 of the HLA-DPB1 are prone to develop beryllium sensitization and eventually, if exposed, beryllium disease [29]. In firefighters and people exposed to photocopy toner dust, clusters of sarcoidosis or a disease indistinguishable from sarcoidosis have been reported [63, 91]. Noninfectious microbial products are capable of inducing autoimmune diseases by an interleukin (IL)-12-dependent pathway. The cytokine-modulating properties of these products convert quiescent autoaggressive T-cells in effector cells capable of transferring immunopathological mechanisms [92]. In line with this observation is the finding that exposure to a respirable bioaerosol containing endotoxin and microbial contaminants during work as a lifeguard at an indoor pool may induce a granulomatous pneumonitis identical to sarcoidosis [1]. Thus, the exposure of susceptible individuals to minute amounts of both organic and inorganic agents may cause diseases indistinguishable from sarcoidosis, lending further support to the hypothesis of an environmental cofactor.

**Clinical features**

Although the onset of the disease is usually insidious and discovered in asymptomatic individuals by routine chest radiography or heralded by constitutional complaints, it will occasionally manifest itself as a medical emergency. Involvement of the eye, heart or central nervous system or the development of hypercalcaemia may require immediate action. More than 90% of sarcoid patients will eventually develop pulmonary abnormalities easily recognizable on chest radiography or by tests of pulmonary function [93].

The chest radiograph is rarely normal; most commonly it reveals bilateral hilar adenopathy and/or diffuse reticulonodular infiltrates in the parenchyma. Lung function tests usually reveal a decrease in lung volumes (vital capacity and total lung capacity), a reduced diffusing capacity and a mildly reduced arterial oxygen tension that may diminish further with exercise. In general, the ratio of forced expiratory volume in one second to vital capacity is normal [94], although sensitive tests reveal airflow limitation [95] and ~20% of the patients present with unspecified bronchial hyperreactivity [96, 97].

An evaluation of transbronchial or open lung biopsies of patients in the early stages of sarcoidosis provides an insight into why the patients suffer from the clinical findings outlined above. The typical findings are those of non-caseating granulomas within the alveolar, bronchial and vascular walls. These granulomas are diffusely scattered throughout the lung parenchyma. In contrast to the granulomas seen in hypersensitivity pneumonitis, the granulomas of sarcoidosis are well-formed, compact aggregates. They are usually of varying age, ranging from highly
cellular lesions to collections with diminishing cellularity, some fibrosis and progressive hyalization. Two characteristic zones can be seen in a typical, well-developed sarcoid granuloma: 1) a central zone or follicle which is tightly packed with cells, composed primarily of macrophages, multinucleated giant cells and epitheloid cells; and 2) a peripheral zone consisting of a collar of loosely arranged lymphocytes, monocytes and fibroblasts [98]. Although many microscopic features may suggest sarcoidosis, the epitheloid granulomas, especially in their earlier stages, are indistinguishable from those of other idiopathic granulomatous disorders or even granulomatous disorders of known origin, such as berylliosis, tuberculosis or hypersensitivity pneumonitis.

Thus, sarcoidosis is best defined in histopathological terms as “a disease characterized by the presence in all of the several affected organs and tissues of noncaseating epitheloid-cell granulomas, proceeding either to resolution or to conversion into hyaline connective tissue” [99]. The clinical diagnosis, however, can only be supported by typical histopathological findings. Pathogenonomic criteria or a diagnostic “gold standard” are absent. Most authorities thus include several clinical, radiological, immunological and histological features in their diagnostic criteria since other disease processes can simulate sarcoidosis in many ways. Occasionally, all of these features may suggest the diagnosis of sarcoidosis in patients later proven to have other diseases [100–102]. Therefore, rigorous efforts have to be made to exclude alternative diagnoses, e.g. tuberculosis, lymphoma and berylliosis [100–105] and patients diagnosed as suffering from sarcoidosis must be regularly subjected to review and further testing [106].

Most patients with diagnosed sarcoidosis will undergo clinical and radiological resolution of the disease over a period ranging from several months to a few years [107]. A few develop a progressive form of the disease which may result in death [108]. In those patients undergoing resolution, subsequent biopsy or material examined at necropsy may reveal changes ranging from complete resolution with no scarring to focal pulmonary scars without evidence of granuloma formation. The fate of the sarcoid granuloma is morphologically well documented. It may appear fresh for months to years, however, the granuloma ultimately resolves, leaving no morphological changes, or undergoes an obliterative fibrosis. The fibrosis starts as a rim of collagen around the granuloma and proceeds in a centripetal fashion until the entire structure is replaced by fibrous tissue. The late stage of sarcoidosis is characterized by an extensive, patchy pulmonary fibrosis and a hypertrophy of pulmonary arteries [1, 98, 99].

The natural course of sarcoidosis is unpredictable in an individual patient. For instance, patients with advanced pulmonary infiltrates and splenomegaly may have spontaneous recovery, whereas others with asymptomatic hilar adenopathy may develop severe disease. Extensive clinical and epidemiological studies performed since 1950 emphasize the generally favourable outcome of sarcoidosis. About 70% of the patients presenting with hilar adenopathy alone (i.e., radiographic Type I) have a spontaneous resolution. In patients presenting with diffuse pulmonary infiltrates with (Type II) or without (Type III) hilar adenopathy this figure is reduced to about 50%. A 40% mortality rate has been observed in those who presented with radiographic signs of fibrosis. In general, the more severe the clinical findings at the time of diagnosis and the more organ systems are affected by the disease, the more frequently adverse courses have been observed. Cutaneous sarcoid frequently indicates chronic and disseminated involvement and the prognosis, in general, is poor in patients with advanced skin lesions [10, 108, 109].

**Immunopathogenesis**

**Alveolar macrophages**

A number of cytokines chemotactic for monocytes is produced by alveolar cells in the course of inflammatory reactions of sarcoidosis and other interstitial lung diseases [110–112], leading to a considerable increase in lavage cellularity with an expansion of the absolute number of alveolar macrophages. The percentage of alveolar macrophages with monocytic appearance is elevated in sarcoidosis and idiopathic pulmonary fibrosis, suggesting a recent immigration of monocytic precursors of alveolar macrophages from the blood [113]. However, those markers characteristic for a monocytic immunophenotype can be acquired by alveolar macrophages in the course of activation [114] and evidence for a local proliferation of alveolar macrophages has been obtained for sarcoidosis [115]. Thus, the question of monocyte immigration has not yet been settled.

The activated state of these cells has been demonstrated by their spontaneous *ex vivo* cytokine production. IL-1, tumour necrosis factor (TNF-α), IL-6, macrophage inflammatory protein-1 (MIP-1), monocyte chemotactic protein-1 (MCP-1) and "regulated on activation, normal T-cell expressed and secreted" (RANTES) have been identified as being released by alveolar macrophages in the course of sarcoidosis [111, 116–121]. This activation of the cells of the monocyte/macrophage lineage is compartmentalized, *i.e.* alveolar macrophages release these mediators spontaneously, whereas the corresponding cells of the peripheral blood are quiescent. Kinetic studies on the transcription of the TNF-α gene revealed that maximal messenger RNA (mRNA) transcription is reached within 2 h after stimulation. Cytoplasmic TNF-α was detected as early as 1 h after stimulation, increasing over the next 2 h to be followed by a decline [122]. Similar experiments with sarcoid alveolar macrophages revealed the transcription of the TNF-α gene at the time of BAL and its downregulation during the next 24 h in cell culture [118]. Thus, the heightened spontaneous TNF-α release seen in active sarcoidosis is a consequence of an *in vivo* activation step just prior to the removal of the cells from the lung, indicating that the eliciting agent resides in the lower respiratory tract. In addition, a priming for an increased release of leukotriene B4 was observed in sarcoid alveolar macrophages, which indicates a different response of these cells to stimulating agents [123]. TNF-α, in particular is released at high concentrations; however, a corresponding cachectin effect is absent in most patients, giving rise to the hypothesis that TNF-α binding or -neutralizing proteins or counteracting cytokines are simultaneously released [124].Soluble TNF receptors (sTNF-R) may be capable of counteracting TNF-α effects
In many pulmonary disorders including sarcoidosis elevated levels of sTNF-R are found in the BAL fluid and increases in the plasma levels of these molecules correlating with disease activity have been observed [127]. Preliminary results obtained by the author’s group demonstrate the presence of increased sTNF-R serum levels correlating with disease activity have been observed [126] and increases in the plasma levels of these molecules [125]. In many pulmonary disorders including sarcoidosis, however, have been complicated by possible stimulations via minor histocompatibility antigens or by intrinsic activities of autologous sarcoid T-cells present in the test. In a different cell-culture system using a method insensitive to histoincompatibilities, sarcoid alveolar macrophages were shown to express increased accessory functions mediated by adhesion molecules, e.g. CD80 [131]. This increased accessory function could only be demonstrated in patients with active sarcoidosis, and not in patients with inactive sarcoidosis or controls. In addition, alveolar macrophages from patients with other granulomatous (tuberculosis, hypersensitivity pneumonitis and Wegener’s disease) and nongranulomatous lung diseases also disclosed increased accessory function of alveolar macrophages, suggesting that this increase is an unspecific stimulation marker of these cells. One mechanism for the induction of CD80 and CD86 is the interaction of MHC class II molecules with the T-cell receptor [132]. Furthermore, increased accessory function can be induced by T-cell-derived cytokines such as IL-2 [133] or interferon-gamma (IFN-γ) (G. Zissel, unpublished observations). This, again, indicates that T-cell activation, possibly by a nominal antigen, is involved in the pathogenesis of sarcoidosis. It is hypothesized that costimulation by CD80 and CD86 influences the differentiation of T-helper (Th)1/Th2 cells as CD80 costimulation leads to more of an activation of Th1 cells, whereas CD86 costimulation induces only Th2 cells [134]. Therefore, increased accessory function of alveolar macrophages influences the composition of alveolar T-cells and the cytokine pattern in the lower respiratory tract.

**T-cells**

Sarcoidosis is associated with an increase in the number of alveolar T-cells and a shift to an increase in CD4+ cells within these cells can be seen. In some cases of sarcoidosis >50% of T-cells with a CD4/CD8 ratio >10 which exhibit markers of activation, such as increased HLA-DR, very late activation antigen-I (VLA-1), and IL-2 receptor expression and capping of the T-cell antigen receptor (TCR), can be observed [4, 135–138]. The T-cells of the granuloma exhibit a layer-like distribution, with CD4+ cells expressing an abundance of activation markers predominantly in the inner area and an accumulation of CD8+ cells with smaller numbers of those markers in the outer area [139, 140].

Alveolar T-cells have been found to release in vitro IL-2 without stimulation in tissue culture [141, 142]. In spite of the systemic nature of the disease only the alveolar T-cells and not those of the peripheral blood spontaneously secreted IL-2. Interestingly, the regulation of the transcription of the IL-2 gene appeared to be normal, indicating a stimulation of the cells in a physiological fashion [88, 137]. This view is supported by the finding of in vitro Bos et al. [138], who demonstrated a capping of the TCR of alveolar T-cells in sarcoidosis, suggesting a recent activation of the cells via this complex. These two phenomena can only be observed in cells of the BAL, indicating that the eliciting agent resides in the lung or that activated cells are attracted to the lung. A similar activation can be assumed for the T-cells of the granuloma since they contain mRNA for IL-2, IL-6 and IFN-γ, which is in line with the detection of these cytokines in lymph node extracts of patients with sarcoidosis [143, 144].

The enumeration of IL-2 receptor (IL-2R)-positive T-cells was considered to be one approach by which to estimate the number of activated alveolar T-cells. Only a moderate increase in IL-2R+ T-cells, with only a few cells going through the S-phase of the cell cycle, was observed [88, 137, 145], suggesting the presence of a small number of activated cells in the alveolar space or a dysregulation in the expression of the IL-2R. Results obtained by an in vitro study with sarcoid T-cells excluded the latter possibility [137]. However, the milieu of the lower respiratory tract generated by type II epithelial cells modulates the reactivity of the T-cells. In the presence of type II epithelial cells, activated T-cells are arrested and do not progress further in the cell cycle. This blockade is reversed when the cells leave this suppressive milieu, e.g. after migration to the lymph node [146]. In fact, in sarcoid lymph nodes an exaggerated lymphocyte proliferation can be observed [147].

Studies with the Kveim reagent revealed that its activity resides within the membrane fragments of alveolar macrophages, thus corroborating the hypothesis that a sarcoid-specific protein is presented by these cells [148]. However, an antigen has not yet been identified and, therefore, several researchers have analysed the usage of the TCR V-region and C-region repertoire in sarcoidosis. The underlying assumption of this approach is that T-cell clones are activated and expanded by the postulated “sarcoid-antigen”, resulting in an increased usage of certain V-region families of the TCR, which can be evaluated by staining or by identifying the V-region mRNA in these T-lymphocytes. Mekel et al. [149] were the first to demonstrate a bias towards an increased usage of the Vβ8 region of the TCR in sarcoidosis peripheral blood and BAL T-lymphocytes, suggesting that T-cells accumulate as a result of external selective pressure, rather than in a random polyclonal fashion or by clonal expansion of one or a few T-cell clones. This observation has been extended to show restricted usage of TCR Vα [150], Vβ [151] and Cβ-chains [152] in BAL and lung parenchyma [153]. However, no clear distinction between polyclonal and oligoclonal T-cell proliferation in sarcoidosis could be made. Other technical approaches have demonstrated an increased clonality in BAL cells without detecting preferred V-region families, leading to the assumption that the major source of T-cells is a polyclonal unspecific accumulation accompanied by a clonal expansion, contributing about 10% of the T-cells [154, 155]. In these studies sarcoidosis was not observed to select certain Vβ-families of the TCR in the detected
clones, thus supporting the hypothesis of an unspecific stimulus. However, KIERS et al. [89] demonstrated an increased percentage of \( \text{V}_\beta^1, \text{V}_\beta^3, \text{V}_\beta^6 \) and \( \text{V}_\beta^8 \) families in intradermal lesions of Kveim skin-tests compared with peripheral blood and that this increase was oligoclonal. These findings are consistent with an antigen-driven T-cell activation and the limited clonality of T-cells could also be demonstrated in sarcoid lung T-cells by analysing the nucleotide sequence of the TCR [156]. The association of this oligoclonality with the course of the disease is demonstrated by the fact that oligoclonality decreases after clinical improvement of the disease either by spontaneous remission or after corticoid therapy [156]. In addition, in some cases an amino acid motif in the \( \text{V}_\beta/J\beta \)-region of the TCR could be identified which had not been described before and might therefore be typical for sarcoidosis [151]. These data suggest that, at least in part, the immune response is elicited by a "sarcoid antigen". In view of the absence of a clonal expansion of one distinct \( \text{V}_\beta \)-family the hypothesis of a superantigen as a possible causative agent of sarcoidosis can be excluded.

FURMAN et al. [151] studied the TCR \( \text{V}_\beta \) distribution in normal lung and blood by quantitative PCR analysis and observed similar distributions in both compartments and that this distribution is relatively stable with only limited changes in a healthy individual over time [157]. In line with this finding are the results of BURASTERO et al. [158] who analysed the TCR \( \text{V}_\beta \) pattern of T-cell clones generated from BAL and peripheral blood and found no significant difference in the TCR \( \text{V}_\beta \) usage in these two body compartments. Thus, the analysis of the TCR \( \text{V}_\beta \) pattern of T-cell clones from sarcoidosis patients will provide insight into immune mechanisms taking place in body compartments other than blood or BAL. In a recent study in the author's laboratory T-cell clones were generated from lung parenchyma, BAL and peripheral blood of patients with sarcoidosis. The analysis of the TCR \( \text{V}_\beta \) pattern of these clones revealed a variability of \( \text{V}_\beta \) family usage of the T-cells in the analysed compartments (fig. 1) and the most prominent changes were observed in the CD4+ cells of BAL. Most interestingly, the TCR \( \text{V}_\beta \) pattern of the compartments BAL and lung parenchyma did not show iden-tical skewing, but exhibited different clonal expansions demonstrating substantial differences in the composition of the T-cell population of these neighbouring compartments [153]. The rise and fall of oligoclonality of BAL CD4+ cells can be observed in berylliosis [159] and that of CD8+ cells in hypersensitivity pneumonitis [161]. More-over, BAL T-cell oligoclonality vanished in patients with sarcoidosis or hypersensitivity pneumonitis after spontaneous resolution or after treatment of the respective dis-order [156, 160]. These observations in interstitial lung diseases of known origin suggest that the skewing of the TCR \( \text{V}_\beta \) families in sarcoidosis is the result of an oligoclonal T-cell activation by a still elusive "sarcoid agent". GREENWALD et al. [161] sequenced the rearrange-ment of the \( \text{V}_\beta/J\beta \) gene segment of the TCR in sarcoidosis patients without identifying any preferences of \( \text{V} \)-region gene usage. Moreover, sequence differences of the rearranged gene segments did not result in changes of the amino acid sequence of the antigen-binding site of the TCR, which provides further evidence that in sarcoidosis T-cells be-come activated by a nominal antigen leading to an oligoclonal T-cell expansion.

In the past few years two functional distinct subsets of CD4+ T-cells have been described by their capacity to release a definite panel of cytokines, first in the mouse [162, 163] and later in the human system [164]. Th1 cells release IL-2, IFN-\( \gamma \) and lymphotoxin and are related to cell-mediated immunity, while Th2 cells release IL-4, IL-5, IL-10 and IL-13 and are related to B-cell help. Both lineages derive from naive Th0 cells, which are able to release the whole panel of cytokines and differentiate into either Th1 or Th2 cells after antigen stimulation, depending on antigen concentration, the affinity of the antigen to the MHC class II molecules and the nature of the antigen. In sarcoidosis, a spontaneous release of Th1 cytokines (IFN-\( \gamma \) and IL-2) by BAL T-cells could be demonstrated...

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**Fig. 1.** T-cell clones generated from different body compartments in patients with sarcoidosis (□□) and controls (□□). The expected range of the \( \text{V}_\beta \) family is also shown (□□□). Clones were generated from: a) peripheral blood, b) transbronchial biopsy, and c) bronchoalveolar lavage. The variable region of the \( \beta \)-chain (\( \text{V}_\beta \)) usage of the T-cell antigen receptor (TCR) was analysed by enzyme-linked immunosorbent assay with antibodies against oligomorphic parts of the TCR characteristic for the different families and indicated as a percentage of all analysed clones. By analysing nine \( \text{V}_\beta \) families about 40% of the T-cell repertoire was screened. The \( \text{V}_\beta \) pattern indicates skewing in the sarcoïd clones similar to that of antigenic disorders, supporting the notion of an antigen-driven selection of the TCR repertoire. (Modified from [153].)
[141, 165], but not the release of Th2 cytokines [126, 166, 167]. Indeed, Bäumer et al. [168] demonstrated that T-cell clones derived from peripheral blood, BAL cells and lung parenchyma of sarcoid patients disclose the entire spectrum of cytokine patterns. Th2-like cells could be demonstrated in all three body compartments, including BAL. From this, one can assume that although Th2 cells are present, they are not activated or even suppressed, which may contribute to the immunopathogenesis by maintaining a cytokine imbalance in the lung. The importance of the cytokine balance for the development of a disease can be demonstrated by an asthma patient with improving asthma (a Th2 disease) during an attack of active sarcoidosis (a Th1 disease) [169].

In addition to the bias described in the usage of the α/β TCR, a bias in the use of the γδ TCR has been observed. In some sarcoidosis patients elevated numbers of γδ TCR positive blood and lavage lymphocytes have been reported [170–172]; a bias for the use of some Vγ chain genes was identified and these cells exhibited signs of recent activation [173, 174]. The observation of increased levels of circulating γδ T-cells has renewed interest in the potential role of mycobacteria in the aetiology of sarcoidosis. However, Tazi et al. [175] demonstrated that the majority of T-cells in lymph node granulomas and in Kveim granulomas of sarcoid patients are α/β-T-cells.

The majority of the findings with regard to sarcoid T-cell response are characteristic of a T-cell-mediated response to antigen and are highly suggestive of the presence of a persistent, poorly degradable antigen or antigens. This concept is further supported by studies of autoimmune and chronic inflammatory diseases demonstrating a compartmentalized accumulation of T-cells with restricted Vγ chain gene usage [176, 177].

Granuloma

Granuloma is a feature of many chronic interstitial lung diseases, e.g. sarcoidosis, hypersensitivity pneumonitis, berylliosis and histiocytosis X. An initial event triggering granuloma formation in diseases of known origin is the deposition of antigenic substances in the lung, as observed in tuberculosis and hypersensitivity pneumonitis. Interestingly, in berylliosis the triggering event seems to be the binding of beryllium to HLA molecules on the surface of the immune cells [178]. The immune system, however, recognizes peptides in the context of self on the surface of antigen-presenting cells and the sole binding of beryllium may not be a sufficiently stimulating event. Therefore, other triggers such as an altered cleavage of self-antigens, caused by a beryllium-induced shift of the specificity of restriction proteases, and subsequent presentation of these new peptides in the context of the MHC, are conceivable. In experimental models such a metal-induced presentation of new self-antigens recognized as nonself by the immune system has been identified as a cause of autoimmunity [179, 180]. In sarcoidosis, however, the initiating agent is not known, but it may be found in the membrane of alveolar macrophages, as demonstrated by a granulomatous skin reaction elicited by membrane fragments of sarcoid alveolar macrophages [148].

Many structurally different agents are known to stimulate the formation of immune granulomas and they share some characteristics. Firstly, in the case of infectious agents their habitat is the macrophage or, owing to their particular nature, they have the propensity to be phagocytosed by macrophages. Secondly, they have the capability to persist within tissues or macrophages, either because the micro-organisms involved are resistant to intracellular killing or because the materials resist enzymatic degradation. Thirdly, without a specific T-cell response immune granuloma cannot be generated and therefore, the inducing agents have to be immunogenic. The unknown aetiolog-ical sarcoidosis-inducing agent should fulfill these three criteria.

Granulomas are structured masses composed of activated macrophages and their derivatives, i.e. epitheloid and giant cells. In contrast to the foreign body-type granuloma (e.g. induced by nonimmunogenic silica), the sarcoid granuloma contains more lymphocytes and at times eosinophils, mast cells and fibroblasts. Sequential analysis of the cellular components of these lesions has demonstrated their dynamic nature. An influx, local multiplication and cell death of immune cells can be observed, most probably governed by inflammatory signals. In immune granulomas, as in sarcoidosis, these signals are likely to be cytokines and cell-cell interactions of lymphocytes, macrophages and their derivatives, and fibroblasts [181]. Blocking CD80 and CD86, molecules mediating the accessory signals of macrophages in T-cell activation [131], by monoclonal antibodies suppressed helminth-induced granuloma formation and cytokine release of T-cells, highlighting the interdependence of these processes in granuloma formation [182].

After phagocytosis of the inducing agent the macrophage releases a number of cytokines which mediate migration of activated lymphocytes and monocytes out of the bloodstream into sites of inflammation. Osteopontin, also known as early T-lymphocyte activation protein 1 (Eta-1), a cytokine released by many cells including lymphocytes and monocytes, is shown to promote macrophage migration and adhesion and is secreted by activated T-cells in association with resistance of mice to infection with obligate intracellular bacteria [183]. Eta-1 was released in high quantities by macrophages immediately after the phagocytosis of M. tuberculosis, but only in minute amounts when phagocytosing inert particles. Normal lung and granulation tissue did not stain positive for Eta-1 but it was identified by immunohistochemistry in macrophages, lymphocytes and the extracellular matrix of pathological tissue sections of patients with tuberculosis or silicosis [184]. The formation of granuloma requires the presence of TNF-α, IL-1, IFN-γ and other cytokines. Animal studies of immune and foreign-body granulomas suggest that IL-1 is important in the early recruitment stages of granuloma formation, while TNF-α may take part in later maintenance or effector functions [185]. This view is supported by the observation that depletion of TNF-α led to a rapid regression of fully developed immune granuloma and suppressed the accumulation of mRNA in macrophages surrounding the granuloma. The latter indicates that TNF-α enhances its own synthesis and release, thus favouring further macrophage accumulation and differentiation leading to bacterial elimination [186]. The requirement of IFN-γ for granuloma formation is demonstrated by the absence of granulomas in IFN-γ gene knockout
mice, which do not respond with a granulomatous reaction after exposure to thermophilic bacteria [187].

The role of T-cells in the development and maintenance of granuloma can be studied in infectious diseases and their animal models. Experimental infection of susceptible mice with *Leishmania major* results in a disseminated, lethal disease and the infected animals respond with CD4+ Th2 cells secreting IL4, IL-5, IL-6 and IL-10, promoting a humoral and suppressing a cellular immune response. In marked contrast, CD4+ IL-2, IFN-γ and TNF-β-releasing Th1 cells are observed in resistant strains which respond with a strong cellular immune reaction. Evidence from human leishmaniosis suggests that the Th1 or Th2 polarized response determines whether subclinical or progressive disease develops [188]. Using mycobacterial and schistosomal antigens Type 1 (IFN-γ and TNF-β dominant) and Type 2 (IL-4 and IL-5 dominant) granulomatous responses can be elicited in normal mice. Knockout of the IFN-γ gene converts the Type 1 response to a response with decreased TNF-β and increased secretion of IL-4, IL-5 and other Type 2 cytokines and eosinophilic infiltration. IL-4 gene knockout exacerbates Type 1 response with compartmentalization of the expected exaggerated IFN-γ release to the lymph nodes and a decrease in IFN-γ transcripts in the lung. Most interestingly, IL-4 gene knockout did not convert Type 2 to Type 1 granulomas [189]. Along this line a Type 1 cytokine pattern has to be expected in tuberculous and sarcoid granulomas. BERGERON *et al.* [190] analysed the presence of mRNA of 16 cytokines in granulomatous lymph node tissue of patients with tuberculosis and sarcoidosis and found a Type 1 response in sarcoidosis and Type 0 response (less polarized to Type 1) in tuberculosis. In addition, they demonstrated that distinct histological features were associated with characteristic cytokine patterns, e.g. neutrophilic infiltration heralded the presence of IL-8 transcripts [190]. Taken together, these studies suggest that a more sophisticated modulation of cytokine expression with other drugs than corticosteroids might be a way of improving the outcome of patients with sarcoidosis and other granulomatous disorders.

### Fibrosis

Granulomas may serve as a focus for fibrosis, with the pattern of fibrosis clearly differing among the various granulomatous disorders. In sarcoidosis a more focal perigranulomatous type is observed. The immune cells composing the granuloma secrete cytokines that attract, stimulate and deactivate fibroblasts, which seems to be dependent on immunological cytokines [182, 191, 192] such as interferon. The latter could be demonstrated by comparing the IFN-γ release of BAL cells from sarcoid lung areas with focal fibrosis with that from areas shown to be free of fibrosis in the computed tomographic (CT) scan [193].

Monitoring the turn-over of cellular matrix in the process of fibrosis should be a way of estimating the status of fibroblasts. Several parameters have been thoroughly evaluated to serve as markers for pulmonary fibrosis, e.g. type III procollagen peptide, collagenase, hyaluronan, and fibrinogen and its degradation products [194–199]. The problem encountered with this concept is that none of the named markers can differentiate between pathological fibrosis and normal tissue turnover in inflammation, as demonstrated by the fact that some markers correlate with parameters of alveolitis [198], although conflicting results have been obtained in longitudinal studies [196, 197]. Thus, at present clinically applicable parameters to monitor or even predict fibrosis do not exist [200, 201].

### Cytokine network

The interactions of the activated cells described above require mechanisms of signal transduction, the cytokine network of which has been analysed in detail as the required methods have been available since the early 1980s. In this complex network, the effect of an individual cytokine varies with the state of activation of the target cell, the presence of other cytokines in the local microenvironment and the ability of the target cells to produce arachidonic acid metabolites.

By the presence of IL-2 and IFN-γ [88, 141, 165] and the absence of IL-4 and IL-5 [126, 190, 204] a functional predominance of Th1 cells can be demonstrated in the lower respiratory tract of patients with sarcoidosis, although the capacity for producing Th2 cytokines seems to be maintained [168]. One key cytokine for the induction of Th1 cells is IL-12, a product of activated macrophages and a strong inducer of Th1 responses. A higher expression of the bioactive cytokine heterodimer (p70) and the mRNA of the regulated p40 subunit were found by MOLLER *et al.* [202] in sarcoid BAL cells compared with controls. This increased level of IL-12 upregulates the development of Th1 cells and amplifies the release of Th1 cytokines, especially IFN-γ.

In the course of sarcoid alveolitis an abundance of cytokines, soluble cytokine receptors and soluble adhesion molecules capable of attracting and activating immune cells and of inducing and maintaining granuloma are released by alveolar macrophages, T-cells and epithelia. Alveolar macrophages release IL-1 and TNF-α [117, 118], pivotal cytokines for the maintenance of granuloma [185, 186]. Alveolar T-cells activated by alveolar macrophage-derived IL-12 release IL-2, IL-16, INF-γ and other cytokines which attract mononuclear cells to the alveoli, activate these cells and induce their proliferation and differentiation [88, 164–167, 203, 204]. In this regard the experimental administration of IL-2 in tumour patients by inhalation is of interest as it revealed that this cytokine activates alveolar macrophages [133] but does not induce further cytokine release [205]. Thus, alveolar T-cells and macrophages activate each other and there is evidence that the cytokine pattern of the alveolitis determines the course of the disease. Elevated levels of IL-2 [206], IL-8 [207–209], TNF-α [40] and MIP-1α [209] were found in patients with progressing disease, which demonstrates that these immunopathological processes determine the clinical course of sarcoidosis. Moreover, by measuring alveolar macrophage TNF-α release it is possible to identify subgroups of sarcoidosis patients who will suffer from progression in the near future [40]. The observation of a coincidence of manifestation of sarcoidosis with IFN-α therapy of cutaneous T-cell lymphoma or hepatitis C further supports the notion of the pivotal role of the cytokine network in sarcoidosis [210].
These phenomena of immune cell activation and cytokine release are compartmentalized and can only be observed in the lung and, although sarcoidosis is considered to be a systemic disorder, no activation of peripheral blood cells could be demonstrated [88, 118, 147, 211]. Using more sensitive methods to detect cytokines in serum or cell culture supernatants an elevated serum level of IFN-γ and an increased release of IL-1β, IL-6 and TNF-α by peripheral blood mononuclear cells was recently observed [212–214]. Moreover, an activation of these cells could be demonstrated by identifying IFN-γ mRNA transcripts [214]. Owing to the rapid turnover of mononuclear cells in the peripheral blood an activation process in this body compartment seems rather unlikely [215, 216].

The human CXC and CC chemokine families of chemotactic cytokines are closely related mediator families which diverged from a common ancestral gene. Chemokines can be produced by an array of immune and nonimmune cells present in the lung. In sarcoidosis, CC (IL-8) and CXC (MIP-1α, MCP-1, RANTES) have been found in BAL cell culture supernatants and BAL fluid. They mediate a major part of monocyte, lymphocyte and neutrophil chemotactic activity and are capable of activating these cells [110, 111, 116, 209, 217, 218]. In line with this notion is the observation of elevated chemokine release in patients with active or progressing disease [207–209]. In idiopathic pulmonary fibrosis and animal models of this disorder a critical role of CC and CXC chemokines in the initiation and maintenance of pulmonary lesions and their angiogenesis could be established [111, 191, 219]. Studies into pulmonary fibrosis taking place in the course of sarcoidosis are still lacking.

At the cell surface, cells shed cytokine receptors and adhesion molecules, and in many pulmonary disorders elevated BAL fluid levels of soluble TNF-RI (sTNF-R), sTNF-RII, sIL-2R and intercellular adhesion molecule-1 (sICAM-1) can be found [126]. These molecules are released by alveolar cells [220, 222] and elevated serum levels related to the clinical course are observed in active sarcoidosis and other chronic inflammatory disorders [221, 223–226]. BAL cell release and BAL fluid content of these cytokines do not correlate with their respective serum levels [221, 224, 227], which might be explained by the absence of a basal membrane leakage or only a limited defect of the basal membrane in sarcoidosis [228, 229] and a release of these molecules in other compartments of the body with easier access to serum [221, 224, 230]. Only limited data are available on the function of these soluble molecules. However, immunosuppressive functions via competition for cytokines or pro-inflammatory functions via prolongation of the cytokine half-life are conceivable. Recent studies indicate a role for sIL-2R in the regulation of Th1/Th2 responses [231] and an antifibrotic capability of sTNF-R [232]. An anti-inflammatory role for the IL-1 receptor antagonist (IL-1RA), an immune-regulatory molecule found in BAL fluid and granulomatous lesions of sarcoidosis patients [190, 233, 234], was established in animal models [235]. In sarcoidosis, however, the role of these soluble molecules has not yet been established.

IL-6 is a pleiotropic cytokine with critical participation in immunity against intracellular infections. Together with other pro-inflammatory cytokines such as IL-1 and TNF-α, it is known to be required for the induction of acute phase reactions composed of fever, leukocyte margination, cortisol release and hepatic production of acute phase proteins. While there is little doubt about the pro-inflammatory nature of TNF-α and IL-1, it has remained unclear whether IL-6 is merely an acute phase reaction-inducing mediator or has further immunoregulatory activities. In sarcoidosis IL-6 is coexpressed in activated alveolar mononuclear cells with TNF-α, IL-1, IL-2 and IFN-γ [120, 121, 126, 167, 207, 208, 212], whereas equivocal results have been obtained regarding its expression in sarcoid lymph nodes [143, 190]. Using IL-6 knockout mice Xing et al. [236] demonstrated that IL-6 is critically required to control the extent of local and systemic inflammation by downregulating the expression of pro-inflammatory cytokine genes and upregulating anti-inflammatory molecules. In chronic inflammation, however, IL-6 promotes T-cell activation and proliferation, B-cell proliferation and antibody production. From this it has to be concluded that the role of IL-6 in sarcoidosis depends on the stage of the disease.

At present, the most extensively investigated deactivating cytokine is IL-10, which inhibits cytokine production as well as proliferation of human monocytes and T-cells [237]. Although several investigators have searched for this molecule, its presence in the alveolitis of sarcoidosis could not be established [202, 238], but equivocal results regarding its gene transcription by BAL cells have been obtained [167, 202] and elevated serum levels have been recorded in sarcoidosis patients [211].

 Transforming growth factor-β (TGF-β) belongs to the superfamily of ubiquitous regulatory proteins which are necessary for cell growth, cell differentiation and regulation of extracellular matrix production. Growing evidence also supports the role of TGF-β as an immunoregulator, exhibiting pro-inflammatory and anti-inflammatory activities [239] and inhibiting the development of Th1 cells [164, 240]. TGF-β was found in supernatants of patients with active disease and a spontaneous remission within 6 months after the investigation, whereas patients requiring therapy or suffering from chronic disease disclosed TGF-β levels that were no different from controls. Furthermore, a strong and significant negative correlation was found between IL-2 and TGF-β production by BAL cells [238]. This suggests an inhibitory role of TGF-β on the IL-2 production of T-cells. In keeping with the literature, it can be concluded from these data that the release of TGF-β by BAL cells is indicative of a mechanism which results in the cessation of inflammatory processes. Whether TGF-β is the key cytokine in downregulating the alveolitis or whether it acts together with other, still unknown mediators requires further investigation.

In this regard it is of interest that the activation of T-cells via CD28 is resistant to the downregulation by TGF-β [242]. In some patients an increased expression of CD80, the ligand of CD28, on alveolar macrophages has been demonstrated, indicating an activation of alveolar T-cells via this pathway [131]. Thus, the course of the disease might be determined by the mode of T-cell activation.

In conclusion, the action of the cytokine network in sarcoidosis can be summarized as follows: an unknown agent activates resident T-cells and macrophages, which subsequently release cytokines (IL-2, IL-12, IL-16, chemokines and IFN-γ), which prime and activate neighbouring cells and are chemotactic for mononuclear cells. The activated cells constitute an alveolitis and the cytokines released...
Disease modifying genes

The HLA complex plays a pivotal role in the immune response by presenting antigens for T-cell recognition and defining the self-epitopes tolerated by the immune system. Distinct HLA class II alleles confer both predisposition and resistance to immune-related disorders. In primary biliary cirrhosis distinct haplotypes confer resistance and others are significantly increased in patients with the disease [242, 243]. For sarcoidosis loose positive and negative HLA linkages have been observed in numerous studies, but a clear-cut linkage disequilibrium with a distinct HLA class II allele has not been found [17, 18, 244]. In Scandinavian patients the clinically defined subgroup of severe chronic disease exhibited an under-representation of HLA-DR3 and an over-representation of HLA-DRw6 [17], which indicates that the course of the disease is modified by HLA class II alleles.

In a longitudinal study by Bredel et al. [245] 122 Scandinavian patients were genotyped for HLA class II alleles and clinically monitored for over 10 yrs. They found a significantly increased frequency of HLA-DR17 (old nomenclature DR3) in patients with nonchronic disease lasting <2 yrs (65 versus 17% in controls). Patients developing chronic disease, however, exhibited an over-representation of DR14 (formerly DR6) and DR15 (formerly DR2). For patients with Löfgren's syndrome 19 out of 19 of DR17+ and two out of four of DR17- patients recovered within 2 yrs. Furthermore, a strong association of HLA-DQB1*0201/0202 with nonchronic and of HLA-DQB1*0503 and HLA-DQ*0602 alleles with chronic disease was observed. Thus, genotyping of these Scandinavian patients allowed a subgrouping of patients into categories with significantly different clinical courses. Unfortunately, these results cannot be extrapolated to other ethnic populations since differences in protection and susceptibility conferring alleles have been observed for other diseases [243] and for sarcoidosis those differences are reported between Caucasian ethnic groups [31] and between Scandinavians and Japanese [246]. Nevertheless, this approach seems applicable for a clinical categorization of sarcoidosis patients.

These studies demonstrate that a predisposition to sarcoidosis is hereditary and implicate genes of the HLA locus as necessary but not sufficient determinants of sarcoidosis. However, it has to be kept in mind that only a small fraction of those who carry the implicated HLA susceptibility alleles develop disease and it is difficult to demonstrate that the HLA associations observed are due to formal genetic linkage between the disease and the HLA locus. The possible role of environmental factors in sarcoidosis and other diseases with a genetic background cannot be denied and the participation of additional genes, not necessarily linked to HLA, has to be tested.

In this context the disease-modifying capabilities of a promoter polymorphism of TNF-α are of interest. At position -308 in the promoter region of the TNF-α gene a biallelic polymorphism has been identified which is associated with variation of TNF-α production in health and disease. Recently, this polymorphism has been correlated with susceptibility to severe disease such as cerebral malaria [247], mucocutaneous leishmaniasis [248], chronic bronchitis [249] and poor prognosis in severe sepsis [250] conferred by the TNFA2 allele. These studies suggest that susceptibility to these diseases may be directly associated with regulatory polymorphisms increasing TNF-α production.

Since cytokine production and in particular TNF-α release by alveolar macrophages is a crucial feature of the immunopathogenesis of sarcoidosis [118], with even prognostic relevance [40], an analysis of the TNF promoter polymorphism was warranted. The TNFα2 allele was shown to confer a six- to seven-fold higher basal and induced TNF-α mRNA transcription than the more frequent allele (TNFA1) [251]. Although its assumed effect of
elevated TNF-\(\alpha\) levels in vivo has not yet been clearly demonstrated, this seems likely because children who developed cerebral complications of malaria were more prone to carrying the TNFA2 homozygote genotype and had higher TNF-\(\alpha\) serum levels [247].

The TNFA1/TNFA2 polymorphism was studied in 101 patients with sarcoidosis and 216 controls and a significant shift to the TNFA2 allele in Löfgren's syndrome was shown (TNFA1/TNFA2 = 0.59/0.41 versus 0.81/0.19 in controls, p<0.01). Comparing Löfgren's syndrome with nonacute sarcoidosis also revealed a significant shift towards the less common TNFA2 allele in Löfgren's syndrome. The entire sarcoidosis cohort, however, did not differ in their TNFA1/TNFA2 allele frequency from control (0.77/0.23). Thus, in contrast to the diseases cited above for patients with sarcoidosis the TNFA2 allele seems to confer a good prognosis [252]. However, in an ongoing study of the author's laboratory the exaggerated TNF-\(\alpha\) release of patients with TNFA2 allele could not be demonstrated, casting some doubt on the pathogenic relevance of this association. Compared with phenotype analysis, such as quantifying alveolar macrophage TNF-\(\alpha\) release, genotype analysis is an indirect measure and provides only categorical data. However, the phenotype may change with either environmental exposure, disease processes or even with sex as the presence of soluble TNF receptors in serum and BAL in sarcoidosis and other interstitial lung diseases has demonstrated [223, 253, 254] and different TNF-\(\alpha\) serum levels were measured in male and female sepsis patients (J. Schröder, unpublished data).

The TNF gene resides in the MHC class III region and the TNFA2 allele was found to be in a linkage disequilibrium with some HLA alleles; among others, in Caucasians with HLA-DR17 [255]. In Scandinavian patients with sarcoidosis HLA-DR17 conferred a good prognosis [245]. Thus, TNFA2 may constitute a passive component of the "Löfgren haplotype", however, on the basis of the available data the opposite cannot be excluded. The increased frequency of the TNFA2 allele in some immune-related disorders may be dependent on its association with HLA-DR17 [256, 257]. In children with manifestations of cerebral malaria, however, an association with HLA class I or class II alleles was not observed [247]. In this context, the notion of ethnic differences in the frequencies of the TNF promoter polymorphism is noteworthy and in a Japanese study the TNFA2 allele was over-represented in patients with sarcoidosis; however, on the basis of the known association between HLA-B and TNFB alleles in the Japanese population this over-representation has to be regarded as extended disease-associated haplotypes of the HLA locus [258]. Thus, in sarcoidosis as in other disorders the interpretation of associations of TNFA and TNFB polymorphisms is difficult owing to linkage disequilibrium between these alleles and HLA class I and II alleles.

Delineated markers

Bronchoalveolar lavage markers

Since BAL probes the compartment of the body most frequently harbouring critical immune mechanisms of sarcoidosis it was assumed that clinical parameters of BAL would be the best approach by which to estimate the inflammatory activity of sarcoidosis and many cytological parameters have been suggested. Follow-up studies, however, revealed ambiguous data and thus the prognostic value of this approach was questioned. Activation markers such as the expression of HLA-DR or IL-2R indicate an activation of BAL cells, but the extent of these phenomena does not allow the separation of patients into groups with different, clear-cut clinical characteristics [135, 137]. Initially, the low numbers of cells expressing those markers was surprising, however, this expectation was based on in vitro studies in which first-order reactions take place [259]. In vivo saturating levels of antigen, helper and T-cells are not provided and in these rate-limiting situations second- or even third-order reactions that generate completely different characteristics of activated cells might dominate. Thus, the transfer of in vitro phenomena of immunoregulation to immunopathological in vivo situations might lead to false expectations and the observed relatively low numbers of BAL cells expressing activation markers might well be a physiological outcome of T-cell antigen interactions with only weak stimulatory capacity. Following those sarcoidosis patients with >40% HLA-DR expressing BAL T-cells revealed 14 out of 15 cases with progressive disease [260], which demonstrates that strong activation processes correlate with an unfortunate clinical course.

A parameter with some prognostic value is the CD4/CD8 ratio of BAL T-cells which, in the case of a strong elevation, indicates a good prognosis and is associated with chronic disease when a lower ratio is observed [261–263]. Other researchers, however, could not establish a clear prognostic value of this parameter [40]. Ambiguous results regarding the prognostic value of the percentage of lymphocytes have been obtained [40, 264–268], but the combination of the percentage of BAL lymphocytes with the spontaneous in vitro IL-2 release of these cells reveals that patients with elevated numbers of IL-2 releasing BAL lymphocytes have the propensity to suffer from progressive disease [206]. Functional parameters obtained by tissue culture of BAL cells or by measuring cytokines in BAL fluid are difficult to obtain in clinical routine but they demonstrate that immunological processes are critical for the clinical outcome.

The observation of heterogeneous TNF-\(\alpha\) release by alveolar macrophages in groups of sarcoidosis patients with active/inactive disease [118], good/poor prognosis [238] or with/without therapy [269] led the author to conduct a follow-up study correlating spontaneous alveolar macrophage TNF-\(\alpha\) release with the course of the disease. The heterogeneous TNF-\(\alpha\) release was reproduced, showing both elevated and normal levels in groups of patients with fortunate and progressing course or with and without therapy. In those patients who had no indication of therapy at the time of BAL an elevated alveolar macrophage TNF-\(\alpha\) release marked a significantly higher risk of deterioration requiring therapy [40]. In those patients with indications for therapy at the time of BAL this parameter showed a trend towards a higher rate of therapy failure in the subgroup with elevated TNF-\(\alpha\) release [40]. Thus, the analysis of alveolar macrophage cytokine release can help to identify subgroups of sarcoidosis patients with different risk patterns for deterioration or failure of therapy. Note, that this information cannot be extracted from pulmonary function tests [40, 269].
Serum markers

In a routine setting sequential BAL are not practical and serum markers are required. Several molecules shed by activated immune cells or epithelia build elevated serum levels which may be used to probe the corresponding immune processes. At present, practical parameters are available for the granuloma burden, T-cell activation and macrophage/macrophage activation.

ACE and lysozyme are molecules secreted by epithelial cells and their serum levels indicate the total body granuloma burden [39]. The sensitivity, specificity and prognostic value of these parameters are low [40, 270, 271] (fig. 3), but the detection of an insertion/deletion polymorphism of the ACE gene, which influences the ACE

level of healthy individuals, opens up the possibility of using genotype-corrected normal values, which may be of greater prognostic value [34–37].

Neopterin, a small, 250 molecular weight metabolite of the guanosine triphosphate pathway, is released by activated macrophages and monocytes [272]. It gauges the inflammatory activity of these cells rather than their activation in the course of building granulomas. As expected, elevated serum levels were found in sarcoidosis and are used to monitor the activity of cells of the macrophage/macrophage lineage [273–275]. No correlation between BAL cell-released TNF-α or IL-6 with serum neopterin could be observed [120], giving rise to the hypothesis that the elevated neopterin levels are sequelae of cell activations in body compartments other than the alveolar space, such as lymph nodes, providing secreted molecules with easy access to the serum. Nevertheless, serum or urine neopterin concentrations proved to be useful clinical parameters to probe the activity of the cells of the macrophage/macrophage lineage in the course of sarcoidosis [273–276].

Numerous serum parameters are suggested to gauge the activity of T-cells. Adenosine deaminase and soluble CD27 are sporadically used to estimate the inflammatory activity of sarcoidosis [277–279], however, of the available parameters soluble IL-2R (sIL-2R) is most frequently used for this purpose. sIL-2R can be found in BAL fluid and serum of sarcoidosis patients and it is released by activated alveolar immune cells [221, 280–282]. In addition to lymphocytes, macrophages are capable of expressing IL-2R upon activation and it has been demonstrated that up to 50% of activated sarcoid alveolar macrophages exhibit increased numbers of IL-2R [283]. The relative contribution of lymphocytes and macrophages to the alveolar lining fluid sIL-2R concentration is not known [221, 283]. The absence of a correlation between alveolar macrophage TNF-α release as marker of cell activation and BAL fluid sIL-2R concentration argues in favour of sIL-2R being a T-lymphocyte marker [118].

A study of our laboratory evaluated the predictive value of serum levels of ACE, neopterin and sIL-2R in patients with newly diagnosed sarcoidosis and only sIL-2R was of significant prognostic value. In the author’s study group, 41.2% of the patients with no indication for therapy at time point of BAL and increased serum levels of sIL-2R experienced disease progression requiring corticosteroid therapy, whereas none of those with normal level did (Group B, fig. 3). In those patients with indication for therapy at time of investigation both outcome subgroups (with and without treatment failure) had, on average, an increased initial sIL-2R. Although it was not significant, a similar trend toward higher risk of relapse could be observed in those patients with indication for therapy (Group A, fig. 3). The predictive values of ACE and neopterin are still under consideration, but in the author’s study groups neither yielded significant prognostic information, although a trend towards unfortunate clinical outcome was observed in those with elevated levels of neopterin (fig. 3) [40].

It has to be kept in mind that none of the above-mentioned parameters can be used to establish a diagnosis or to find an indication for therapy, as elevated levels are found in a number of diseases and inflammatory activity may not be the therapeutic target. Once the diagnosis of

Fig. 3. – Prognostic value of serological parameters of disease activity of sarcoidosis. a) Sarcoidosis patients with indications for therapy (group A) and b) without indications for therapy (group B) were divided into subgroups either with high ( ) or normal ( ) levels of the investigated parameters. Relapses during tapering of corticosteroids or after cessation of therapy in group A, or progressive disease requiring therapy in group B, served as endpoints as determined by a follow-up, and are given as percentages on the ordinates. ACE: angiotensin-converting enzyme. The numbers of relapses or cases of progressive disease within the total number of patients in each subgroup are given at the tops of the bars. Sarcoidosis patients with no indication for therapy and high levels of soluble interleukin-2 respecr (sIL-2R) had a significantly greater risk of disease progression (*: p=0.017) than those with a normal sIL-2R level. In both groups, patients with a high serum level of neopterin had a greater risk of relapse or disease progression, although this difference was not statistically significant. (Reproduced from [40] with permission.)
sarcoidosis has been made, the inflammatory activity of some immunopathogenetic processes can be monitored by the use of these serum parameters.

Treatment

Corticosteroids

Because of the variable course of sarcoidosis and potential for spontaneous remissions, indications for therapy are still controversial. Fortunately, many patients will not require treatment because the symptoms are not disabling and frequently remit spontaneously. M. Sones and associates and Sitzbäch, the first to report the remedial effects of short-term corticosteroid therapy of sarcoidosis, observed histological improvement parallelizing other signs of improvement and concluded that this therapy prevents progression of the disorder, although, at the same time, they noted a high rate of relapses after cessation of therapy (reviewed in [284]). Many open and placebo-controlled studies questioned the conclusion of the salutary effects of corticosteroids on the course of sarcoidosis. A major obstacle of those studies is the inability to predict reliably which patients will spontaneously recover and which will deteriorate, and much controversy exists about the criteria for measurement of success. However, extensive clinical experience favors treating patients with severe pulmonary or extrapulmonary disease.

Corticosteroids are clearly necessary for life-or sight-threatening organ involvement, e.g. ocular, cardiac, central nervous system, hypercalcaemia or disfiguring cutaneous involvement. Studies used to support the conclusion that corticosteroids are of no benefit gave data which clearly demonstrate the opposite. After a three-month trial Israel et al. [285] documented an improvement in 53% of treated patients with radiological types II and III versus 16% in the control group. A follow-up study of 5 yrs, however, failed to document a benefit. Similarly, Zhao et al. [286] treated 183 patients with all radiological types, presumably including asymptomatic patients, and observed an 11% deterioration in the placebo group but only 5% in the treatment group. In a recent study from the UK, asymptomatic patients with persistent radiographic infiltrates were randomized to receive either prednisolone for 1 yr or no therapy. Both groups were followed for 5 yrs and a 9% higher vital capacity, lower rate of symptoms and improved chest radiographic findings were recorded for the treatment group [287]. This can be taken as good evidence that corticosteroids attenuate or even avert loss of pulmonary function in patients with radiographic types II and III, even in asymptomatic patients.

From a theoretical point of view a beneficial effect of corticosteroids can be assumed since the administration of this drug effectively suppresses the process of alveolar T-cell IL-2 release and the proliferation of these cells [203]. These are two pivotal characteristics of the alveolitis of sarcoidosis and the IL-2 release is linked with disease progression [206]. In a prospective study by Hunninghake et al. [107] only sarcoidosis patients with evidence of recent deterioration of lung function or severe extrapulmonary disease were treated with corticosteroids for 12 months. Finding the indication for therapy on the basis of these clinical criteria, it was demonstrated that treatment prevented deterioration or induced improvement in lung function. Thus, the ongoing inflammatory processes causing deterioration of lung function can be obstructed and further deterioration can be prevented. (For detailed specifications of the standard of therapy the reader is referred to the literature [1, 288].)

Alternative drugs

For patients with contra-indications for corticosteroids or corticosteroid-recalcitrant sarcoidosis a variety of other anti-inflammatory or immunosuppressive approaches have been evaluated in uncontrolled studies or case reports. For methotrexate [289], azathioprine (Müller-Quernheim et al., unpublished data) and cyclosporin A [290] a corticosteroid-sparing effect can be accepted and its use is suggested in patients who need prolonged therapy with high doses of corticosteroids [291]. For detailed recommendation regarding doses and mode of therapy the reader is referred to clinical reviews [288, 291, 292].

Although cyclosporin A is effective in vitro in suppressing the exaggerated activation of sarcoid alveolar T-cells, it does not do so in vivo, as demonstrated in a study using this drug as a monotherapeutic agent [293]. In contrast, for methotrexate some usefulness as a monotherapeutic drug capable of replacing low-dose corticosteroids was demonstrated and its capacity to suppress immunopathological processes of sarcoidosis such as release of TNF-α and radical oxygen intermediates was recorded [294]. A corticosteroid-sparing effect of methotrexate was observed in a larger series and in some patients prednisolone was discontinued, while methotrexate maintained remission [289]. In a similar way it could be demonstrated that the combination of azathioprine and prednisolone allowed the reduction of corticosteroids, while the suppression of alveolar leukocytes IL-2 and TNF-α release endured and the clinical improvement was sustained (Müller-Quernheim et al., unpublished data). The described concept of the immunopathogenesis of sarcoidosis provides a tool which allows the gauging of the effect of a treatment strategy on critical disease processes that might support or discourage the strategy under scrutiny.

Pentoxifylline inhibits both the primary proliferative response of T-cells in the mixed lymphocyte culture [295] and the endotoxin-induced TNF-α release on the transcriptional level both in vitro and in vivo [296, 297]. The release of IL-12, a mediator promoting the Th1-type of T-cells characteristic for the alveolitis of sarcoidosis, is suppressed by pentoxifylline in a way independent of other known IL-12 inhibitors [298]. Moreover, in combination with dexamethasone, an over-additive effect in the inhibition of in vitro stimulated IL-12 [298], IL-2, IFN-γ and TNF-α [299] release was observed, which suggested that pentoxifylline might be a drug capable of downregulating the exaggerated cytokine release of sarcoidosis. Indeed, in an open clinical study, 11 out of 18 patients with sarcoidosis treated with pentoxifylline monotherapy improved and in patients with corticosteroid-resistant disease, the addition of pentoxifylline allowed the reduction of prednisolone doses in two cases and even the discontinuation of prednisolone in another case, while the remission achieved with the combination was sustained [300].
In 1965, Sheskin [301] reported 6 cases of acute, severe leprosy reactions in which symptoms resolved quickly and dramatically after the use of thalidomide as a sedative. Recent investigations revealed that thalidomide is a drug capable of selective downregulation of several pro-inflammatory cytokines critically involved in the pathogenesis of sarcoidosis and other granulomatous disorders. It inhibits the in vitro release of IL-12 [302] and TNF-α [303] and acts on alveolar macrophages [304]. Thalidomide-induced inhibition of IL-12 production was additive to that induced by suboptimal inhibiting doses of dexamethasone, giving rise to the hypothesis that the administration of thalidomide is another corticosteroid-sparing option in the treatment of sarcoidosis. This is supported by a recent case report of successful treatment of cutaneous sarcoidosis with thalidomide [305].

Conclusions

It is becoming increasingly clear that the action of any individual cytokine is neither completely beneficial nor totally detrimental to the host. Rather, a fine balance of cytokine production and regulation must be maintained to ensure that the host can effectively respond to invading micro-organisms or other disturbances of homeostasis without compromising host well-being in the process. Although the aetiology of sarcoidosis remains elusive, better understanding of the regulation of the network of inflammatory cells and cytokines provides a rationale for designing markers of inflammatory activity of the disease and new therapeutic interventions to prevent the damaging immune-mediated pathology.

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