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SARCOIDOSIS: INFLAMMATORY MECHANISMS AND MARKERS OF ACTIVITY

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ABSTRACT

Sarcoidosis is a disease characterized by an accumulation of CD4+ T-lymphocytes in affected organs. Previous findings of an association between the HLA-allele DR17 and an accumulation in bronchoalveolar lavage fluid (BALF) of CD4+ T-lymphocytes expressing the T-cell receptor (TCR) V gene segment AV2S3, is in line with an aetiological hypothesis claiming that sarcoidosis is elicited by a specific antigen(s) in genetically predisposed individuals.

In the present study immunological features of sarcoidosis were investigated in HLA-DR17 positive patients with active and clinically resolved disease. Besides, the influence of a polymorphism in the angiotensin-converting enzyme (ACE) gene as well as apoptotic functions in lymphocytes from sarcoidosis patients were investigated.

The study revealed differences between DR17 positive and DR17 negative sarcoidosis patients in inflammatory parameters in BALF and serum.

No associations were detected between the ACE gene polymorphism and either susceptibility to sarcoidosis, outcome of the disease or HLA-DR alleles of prognostic interest.

A resistance to apoptosis, paralleled by an increased caspase-3 activity, was detected in BALF lymphocytes from sarcoidosis patients but not from healthy controls. The consequences of this finding remain elusive.

Clinical resolution of sarcoidosis in HLA-DR17 positive patients was associated with normalization in AV2S3+ CD4+ T-lymphocytes in BALF and a decrease in markers of disease activity in BALF and serum.

In DR17 positive patients a difference was found between patients with active and clinically resolved sarcoidosis regarding expression of activation markers, including markers delineating T regulatory cells, by CD4+ T-lymphocytes from BALF and peripheral blood.

In conclusion, immunological differences were found between patients positive and negative for DR17 and between DR17 positive patients with active and resolved disease. The normalization in AV2S3+ lymphocytes strengthens their involvement in the pathogenesis of sarcoidosis.

Keywords: sarcoidosis, HLA class II, T-lymphocytes, CD4-CD8 ratio, T cell receptor, angiotensin-converting enzyme, apoptosis

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LIST OF PAPERS

This thesis is based on five original papers listed below. In the text these papers are referred to by their Roman numerals.

I. **Planck A**, Eklund A, Grunewald J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2001; 18: 64-69.

II. **Planck A**, Eklund A, Yamaguchi E, Grunewald J. Angiotensin-converting enzyme gene polymorphism in relation to HLA-DR in sarcoidosis. *J Int Med* 2002; 251: 217-222.

III. Stridh H, **Planck A**, Gigliotti D, Eklund A, Grunewald J. Apoptosis resistant bronchoalveolar lavage (BAL) fluid lymphocytes in sarcoidosis. *Thorax* 2002; 57: 897-901.

IV. **Planck A**, Eklund A, Grunewald J. Markers of activity in clinically recovered HLA-DR17 positive sarcoidosis patients. *Eur Respir J*. In press.

V. **Planck A**, Katchar K, Eklund A, Gripenbäck S, Grunewald J. T-lymphocyte activity in active and clinically recovered HLA-DR17 positive sarcoidosis patients. Manuscript.

ABBREVIATIONS

ACE	Angiotensin-converting enzyme
Ag	Antigen
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BHL	Bilateral hilar lymphadenopathy
CD	Cluster of differentiation
CDR	Complementarity determining region
CCR	CC chemokine receptor
ELISA	Enzyme-linked immunosorbent assay
EN	Erythema nodosum
EBV	Epstein-Barr virus
FEV1	Forced expiratory volume in one second
HHV-8	Human herpesvirus 8
HLA	Human leukocyte antigen
HRCT	High-resolution computed tomography
IFN	Interferon
IL	Interleukin
mAb	Monoclonal antibody
MCP	Monocyte chemotactic factor
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
PB	Peripheral blood
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PFT	Pulmonary function test
SACE	Serum activity of angiotensin-converting enzyme
sIL2R	Soluble interleukin-2 receptor
TCR	T-cell receptor
TGF	Transforming growth factor
Th	T-helper
TNF	Tumor necrosis factor
Treg	Regulatory T-lymphocytes
VC	Vital capacity

A SURVEY OF SARCOIDOSIS

Glimpses from the past

Sarcoidosis is a derivate from the term sarcoid that was created by the Norwegian dermatologist Caesar Boeck in the late 19th century for cutaneous lesions, consisting of accumulated epithelioid and giant cells, which he thought resembled sarcoma [11]. Boeck also noticed impaired tuberculin reactivity in sarcoid patients, thus an early observation of the involvement of immune mechanisms [12]. The very first description of sarcoidosis, presented two decades earlier by Jonathan Hutchinson 1877, was also based on skin manifestations [58]. Subsequently the disease was discovered in other organs and the Swedish dermatologist Jörgen Schaumann suggested the systemic nature of what he called "lymphogranulomatosis benigna" during the first decades of the 20th century [133]. In the beginning of the 40ths the Norwegian physician Ansgar Kveim discovered that intradermal inoculation of tissue obtained from sarcoid lymph nodes in other sarcoidosis patients elicited a granulomatous reaction. The skin test, known as Kveim-Siltzbach after further development by Louis Siltzbach, has been used worldwide for diagnosing sarcoidosis until recently when usage has declined due to fear of infections and questioning of validity of test material. The mechanisms behind the Kveim-Siltzbach reaction however remain elusive [95]. A thrilling hypothesis is that "the truth is in there", *i.e.* that the extract contains sarcoidosis specific antigen(s). The search for this antigen has though hitherto been fruitless. A younger colleague to Schaumann at St Göran's hospital, Sven Löfgren, a pulmonary physician, based his thesis on a systematic survey of the combination of bilateral hilar lymphadenopathy (BHL), erythema nodosum (EN) and arthropathy [78]. This presentation, known as Löfgren's syndrome, is further described under clinical presentation.

Development of flexible bronchoscopy in the late 60ths [59] followed by usage of bronchoalveolar lavage (BAL) in the mid 70ths [116] has revolutionized the possibilities to characterize the inflammatory reaction in pulmonary sarcoidosis [55, 56, 90]. Numerous analyses of bronchoalveolar fluid (BALF) have also been performed in search for a sarcoidosis specific agent, however the nature of the eliciting agent remains a quiz [133]. The mystery remains, but on a higher level.

Epidemiology

Sarcoidosis appears all over the world, though the incidence differs between populations [102, 107, 133]. Genetic prerequisites is an important reason for these differences, for instance sarcoidosis is reported to be four times more common in black compared to white subjects in an American study [119]. Other explanations may be due to environmental factors but also to possibilities to set the diagnosis and incidence of differential diagnoses [133]. A source of error is also the high frequency of asymptomatic cases, 41% in a review of sarcoidosis in the Nordic countries [86], which may lead to an underestimation of the disease. Two features can nevertheless be distinguished; most cases of sarcoidosis appear between 20 and 40 years and both sexes are approximately equally affected although a slight female dominance is frequently reported [102, 107, 133].

Some of the highest prevalence figures of sarcoidosis are reported from Scandinavia [16, 86, 95]. In a Swedish study, principally based on radiographs performed in general health surveys, the estimated yearly incidence of sarcoidosis was $19/10^5$ [47]. If this estimation is correct, has not changed over time and is equal all over Sweden, it implies that 1476 persons

in Sweden get sarcoidosis each year (based on population >14 year, 31th Dec 2001 [112]). In correspondence with other Scandinavian reports a second incidence peak was observed in females between 45 and 65 years old. This second peak leads to a minor female dominance among Scandinavian sarcoidosis patients [47, 86]. A recent reminder that sarcoidosis is not restricted to younger persons is results from an American case-control study, the ACCESS study, where one third of the patients were 50 years or older [8]. Similar to the Scandinavian situation, the American study revealed a female dominance in patients 50 years and older.

Aetiology

Differences in incidence between ethnical groups and familial clustering indicate that genetic factors influence the susceptibility for sarcoidosis [14, 119]. Others studies though report clustering of sarcoidosis in non-relative subjects who is sharing the same milieu, thus indicating an environmental cause [26, 48, 67, 106]. Furthermore, the immune response in sarcoidosis resembles the response elicited by foreign agents [25, 90, 102]. Taken together these observations have led to the hypothesis that sarcoidosis is elicited by an environmental agent(s) in genetically susceptible individuals [133]. Besides affecting the susceptibility to sarcoidosis, genetic influence may also influence the clinical course [84, 102].

Candidate genes are searched for among those involved in antigen detection and immune regulation, exemplified by those encoding HLA molecules and cytokines, but other genes have also gained some interest such as the angiotensin-converting enzyme (ACE) gene [84, 102].

The long list of agents that have been proposed to elicit sarcoidosis (reviewed in [84]) does not only reflect the numerous attempts to solve an intriguing question but also the fact that granuloma formation reflects a common response against several infectious, organic and inorganic agents [102]. Some genetic and environmental candidates are presented in Table I.

Gene locus	**Infectious agents**	**Non-infectious agents**
HLA-class I [44, 60, 82]	Mycobacteria [81]	Talc [29, 42]
HLA-class II [1, 44, 60, 82]	*P. acnes* [61]	Aluminum [21]
T-cell receptor [30, 38, 70, 90]	*C. pneumoniae* [114]	Pine tree pollen [19]
TNF-α [73, 126, 136]	*B. burgdorferi* [53, 62]	Clay [133]
IL-1 [120]	*R. helvetica* [103]	
CCR2, CCR5 [108]	HHV-8 [22]	
ACE [31, 80, 109]	EBV [133]	

Table I. *Gene loci and agents proposed to be involved in the pathogenesis of sarcoidosis or a closely mimicking disease. Abbreviations, see* **ABBREVIATIONS**.

Diagnosis

The diagnosis of sarcoidosis is based on a typical clinical picture, including chest radiographs if applicable, together with histological proof of noncaseating epithelioid cell granulomas and exclusion of other, mimicking diseases [133]. In the absence of histological

proof, signs strongly supportive for sarcoidosis are Löfgren's syndrome [133] and a ratio between CD4+ and CD8+ T-lymphocytes in BALF >3.5-4.0 [17, 145].

Several infections, hard metals and foreign particles may give rise to granulomas at present not possible to distinguish from those seen in sarcoidosis. A similar clinical and histologic picture may also be seen in other interstitial pulmonary diseases and inflammatory disorders of autoimmune or unknown origin. Important differential diagnoses are lymphomas and other malignancies that may elicit granuloma formation, so-called sarcoid reactions, in adjacent lymph nodes [102, 133].

Clinical features

Sarcoidosis may present with various symptoms, a heterogeneity that reflects the variation of organs that can be affected [133]. In addition to organ specific symptoms, many patients reveal unspecific signs such as fatigue, fever and anorexia [24]. Some problems may be related to hypercalcemi, which is found in up to 10% of the patients [133], though the majority of these unspecific symptoms remain unexplained. Interestingly, recent findings indicate that small fibre neuropathy is common in sarcoidosis, a condition that may give rise to autonomic dysfunction followed by unspecific symptoms [50].

In many patients though symptoms are lacking and the disease may be discovered by chance [47, 86]. The lung constitutes the most frequently involved organ and at least 90% of the patients reveal chest x-ray abnormalities [107]. Additionally, sarcoidosis patients with normal chest x-ray may still reveal signs of pulmonary involvement when examined with BAL [40, 140].

However, pulmonary symptoms such as dry cough, sometimes caused by bronchial hyperactivity [150], chest pain and shortness of breath may be less apparent than extrapulmonary symptoms, especially in patients with an acute disease onset. In contrast, pulmonary symptoms are frequently prominent in patients presenting with a more insidious disease onset.

Other organs commonly affected in sarcoidosis are skin, eyes, lymph nodes, nervous system and the heart [133]. The frequency for each organ involvement differs between ethnical groups, again indicating a genetic influence. For instance, eye involvement is more common in Japanese [110] while the skin manifestation lupus pernio is more frequent in West-Indians [131] and blacks [102].

A common presentation of sarcoidosis in Caucasians is Löfgren's syndrome: BHL on chest x-ray, fever, EN and/or arthropathy [78, 79]. EN usually appears as red, tender, subcutaneous nodules on the lower limbs and constitutes an example of a reactive, non-specific skin manifestation. Besides sarcoidosis, EN may be associated with infections caused by haemolytic streptococci and *M. tuberculosis*, some drugs but also with other inflammatory diseases of unknown aetiology such as inflammatory bowel diseases [115]. It is speculated that EN and arthropathy in Löfgren's syndromes share the same role in the natural history of sarcoidosis though EN is more common among female patients while the arthropathy is dominating in men [124]. Circulating immune complexes are suggested as the aetiology of EN [45] though the absence of circulating immune complexes in some other disease conditions with EN suggests that other mechanisms may be involved [104]. Such mechanisms may also include a genetic disposition since associations have been reported between EN in sarcoidosis and HLA-B8/ DR3 [44] and recently with a polymorphism in the promotor region

for TNF-α [73, 126]. Interestingly, the association with the uncommon TNF A II allele was only detected in sarcoidosis patients with EN but not in those with other causes of EN.

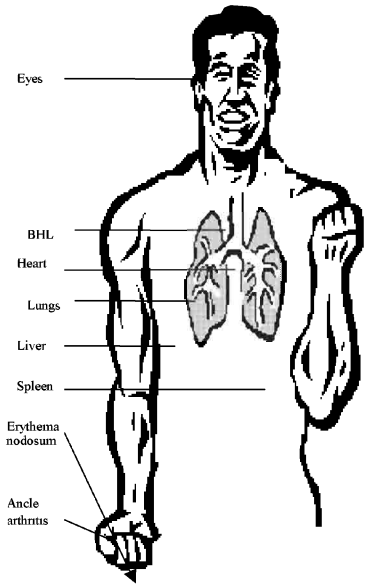

Figure 1. *Some organs that may be involved in sarcoidosis.*

Radiographic feature and pulmonary function tests (PFT)

Frequently used staging systems of sarcoidosis are based on conventional chest x-ray. The staging scale is divided into four to five steps where stage 0 represents a normal chest x-ray without radiographic pathology, stage I and II: BHL without respectively with parenchymal infiltration, stage III: parenchymal infiltration without BHL. An additional stage, stage IV, reflecting signs of pulmonary fibrosis, is sometimes used and has recently been adopted in a statement document on sarcoidosis [133]. High-resolution computed tomography (HRCT) constitute a valuable complement to chest x-ray in particular for differential diagnostic purposes, detection of pulmonary complications and for distinguishing between active inflammation and fibrosis [13, 93]. Conventional chest x-ray though remains a standard tool for monitoring the clinical course in sarcoidosis and contributes with information regarding the prognosis [102, 133].

Although pulmonary function tests in sarcoidosis patients may reveal both restrictive and obstructive abnormalities, lung volumes are frequently within normal ranges despite chest x-ray changes [16]. Examination of diffusion capacity and arterial oxygen tension on exercise is suggested to be more sensitive methods for detection of early pulmonary dysfunction [63, 85]. Interpretation of impairment in pulmonary function may however be difficult since PFT do not distinguish between reversible inflammation and irreversible fibrosis. Nevertheless, serial examination of lung volumes and gas exchange consist important tools in the follow-up of individual patients [102].

Disease activity

The term disease activity in sarcoidosis implies that patients present signs of either ongoing inflammation and/or granuloma formation and/or a fibrosing process [16]. The best-evaluated methods for estimating activity are based on clinical signs together with chest x-ray and PFT. Numerous biochemical and immunological markers obtained from serum and BALF have been suggested to be useful for this purpose as well [16]. Activity markers investigated in the context of this thesis are briefly described in the following two sections.

Serum parameters

Ever since Lieberman [77] discovered an increased activity of **angiotensin-converting enzyme** in serum (SACE) from sarcoidosis patients, SACE has become one of the most frequently analyzed parameters in sarcoidosis worldwide. Originating from the epithelioid cells, SACE in sarcoidosis is believed to reflect the granuloma burden [32, 94]. Although ACE and the peptide it activates, angiotensin-II, exert immunomodulating properties their role in sarcoidosis remains elusive [143]. Besides sarcoidosis, increased SACE may be detected in other diseases such as berylliosis, asbestosis and silicosis which restricts its diagnostic usage [4]. The sensitivity for SACE in sarcoidosis is also rather low, about 57% [135]. A decrease in SACE is frequently detected upon treatment with corticosteroids [4]. Normal activity has also been found initially in patients presenting with erythema nodosum [41], thus a normal SACE does not exclude active sarcoidosis.

Two alleles of the ACE gene can be distinguished depending on the presence (I) or absence (D) of a 287 base pair DNA fragment [117]. This polymorphism implies the existence of two alleles, I and D, that can be combined into three possible genotypes, II, ID or DD. The DD genotype has been reported to be overrepresented in some subgroups of sarcoidosis patients [31, 80] as well as associated with a poor prognosis [109]. Since SACE in healthy subjects is strongly influenced by this I/D polymorphism an increased sensitivity may be achieved if this genetic impact is taken into consideration [129, 137].

Neopterin is a small pteridine derivate which is accumulated and released from macrophages upon IFN-γ stimulation [54], thus serum levels may constitute a reflection of the IFN-γ activity. Neopterin itself has been reported to be involved in immune reactions through activation of the transcription factor NF-kB [49, 146]. In sarcoidosis though, investigators of neopterin have been focusing on its role as an easily obtained marker for inflammatory activity in serum [27, 51] or urine [74, 113].

Patients with sarcoidosis and various other diseases associated with lymphoid activity have increased serum levels of **soluble IL-2 receptor** (sIL2R) [128]. Soluble IL2R probably originates from activated alveolar immune cells and has been proposed to be a marker for T-lymphocyte activity [95]. Besides reflecting disease activity in sarcoidosis, contradicting results concerning the predictive role for serum levels of sIL2R have been presented. Increased levels were prognostically unfavourable in one study [148] while in another they rather reflected a favourable course (discussed in [96]).

BALF parameters

An accumulation of activated, HLA-DR expressing and IL-2 producing **lymphocytes** constitute a characteristic feature of the alveolitis in sarcoidosis [55, 123]. The clinical and

prognostic relevance of the degree of the lymphocytosis however remains controversial since contradicting results are achieved from different studies [66, 75, 142, 144]

A frequently used measure of the alveolitis in sarcoidosis is the relation between CD4+ and CD8+ T-lymphocytes, the **CD4/CD8 ratio**, in BALF. The ratio in sarcoidosis is often increased, sometimes exceeding 10, and has a diagnostic relevance [17, 145]. However, normal ratios are frequently found [145] and though a negative CD4/CD8 ratio is rare, it by no means excludes sarcoidosis [3]. Results from several studies indicate that an increased BALF CD4/CD8 ratio is prognostically favourable [23, 142, 144].

Albumin is another suggested marker for the inflammatory intensity in the bronchoalveolar space and is reported to correlate with other markers of alveolitis in sarcoidosis [91]. Other soluble components that are increased in BALF are **fibronectin** and **procollagen-IIINterminal peptide** [10]. Because of their involvement in tissue turnover there have been expectations that these molecules could be used as predictors for fibrosis. Unfortunately though, no clear correlation between these molecules in BALF and development of pulmonary fibrosis in sarcoidosis has been presented [95].

Prognosis and treatment

The prognosis of sarcoidosis differs from spontaneously resolving disease to a chronic course with development of irreversible organ damage secondary to fibrosis [87, 102, 133]. Mortality rate in sarcoidosis is reported to be between 1-5% in different populations and is most often caused by progressive respiratory insufficiency and cardiac or neurological involvement [102, 133]. Treatment options are based on suppressing the inflammatory reaction, today preferentially with long-term oral corticosteroids. Although the initial response to corticosteroids often is good with symptom relief and radiographic improvement the long-term effect remains uncertain [57, 102, 133]. Furthermore, treatment with corticosteroids for a longer period may be associated with serious side effects [57]. Less harmful, more effective and specific treatment options are therefore requested. Inhaled corticosteroids [111, 132] and TNF-α inhibitors [7] are examples of interesting treatment strategies, still though these alternatives need further evaluations.

Since a majority of patients nevertheless recovers spontaneously, reliable markers that predict the clinical course are useful for avoiding unnecessary treatment. The initial clinical presentation reveals information of predictive importance. A favourable prognosis is often seen in patients with an acute disease onset, as Löfgren's syndrome, and stage I disease. In contrast, insidious disease onset, involvement of several organs and advanced chest x-ray stages may predict a progressive and chronic disease [133]. However, exceptions from these observations exist and numerous attempts have therefore been made to find biochemical markers in serum and BALF that can be used as prognostic markers [23, 66, 133, 144, 148]. Some of them are briefly discussed in the context of activity markers. Finally, genetic analyses may yield prognostic information in some populations [9, 82, 109].

Immunopathogenesis

Analyses of BALF obtained from patients with active, pulmonary sarcoidosis have given much information concerning the inflammatory reaction in sarcoidosis [38, 55, 90, 123]. Typically there is an accumulation of activated T-lymphocytes, preferentially of the CD4+ subset, and of macrophages. Other features are increased immunoglobulin production by hyperactive B-lymphocytes and formation of immune complexes [43, 133]. Release of

cytokines and other soluble substances as well as expression of surface molecules by accumulated immunocompetent and endothelial cells maintain the reaction [2, 89, 95]. Events in this inflammatory milieu subsequently lead to creation of the pathological hallmark of sarcoidosis, the noncaseating epithelioid cell granulomas [89, 95, 133]. Granuloma formation is a well-known strategy by the immune defence in the protection against infectious or other hazardous antigens [2, 133] and a hypothesis is therefore that the granulomas in sarcoidosis reflect a response against an as yet unknown antigen.

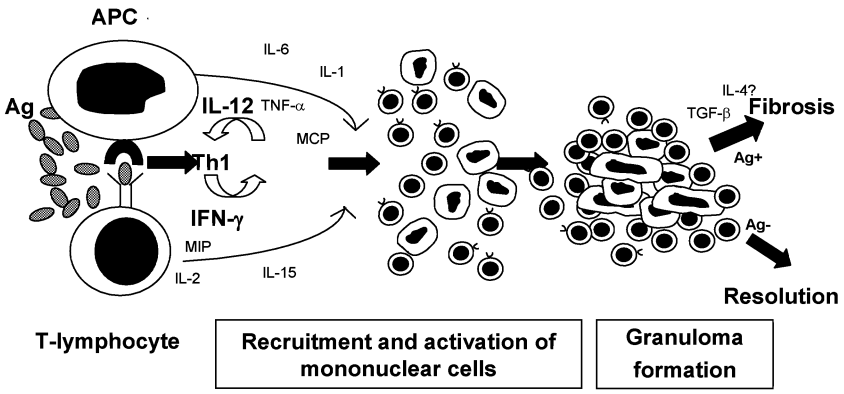

Figure 2. *A hypothetical model of the pathogenesis of sarcoidosis. Abbreviations, see* **ABBREVIATIONS** *(Revised from Moller [89]).*

T-cells

The CD4+ lymphocyte is believed to play a central role as a director of the immune response in sarcoidosis [55, 89, 95] and studies of this celltype have increased our understanding of the underlying immune mechanisms. The cytokine profile expressed by CD4+ T-lymphocytes in sarcoidosis has revealed a deviation towards a Th1 response, *i.e.* with elevated IFN-γ and IL-2 levels, according to the Th1/Th2 paradigm presented by Mosmann *et al* [92, 95, 118]. Th1 cytokines mediate macrophage activation, lymphocyte proliferation and other functions involved in the cellular immune defence and are often, but not invariably, seen in responses that lead to granuloma formation [89].

T-cells recognize antigens through a highly specific receptor bound to its surface. This receptor (TCR) is a heterodimer of two polypeptide chains, α and β. Each chain consists of a constant and a variable region. The variable region contains the antigen binding site, which is created through rearrangement procedures of several gene segments; V, J or V, J and D for the α and β chain respectively. The diversity possible to achieve by rearrangement of these gene segments is enormous, estimated to exceed 10^{15} [20].

Monoclonal antibodies (mAb) directed against a certain V gene segment in part responsible for antigen recognition may reveal a preferential usage of specific gene segments.

A restricted usage of certain V gene segments in a T-lymphocyte population may reflect a specific reaction against an environmental exposure [127].

In 1988 Moller *et al* found a restricted usage for the TCR Vβ8 region of T-lymphocytes obtained from BALF and blood in sarcoidosis [90]. This study was soon followed by additional reports of restricted usage of other Vβ or Vα regions in sarcoidosis [30, 38, 70, 130]. These findings suggest a reaction towards a specific antigen.

The HLA-molecule

The α/β TCR recognizes antigens in the context of a MHC molecule, in humans named HLA-molecules, a feature called MHC-restriction [149]. Each individual expresses several HLA molecules encoded by different gene segments. Depending on structure and function these molecules are divided into two classes; HLA-class I; -A, -B, C and class II; HLA-DR, DP, DQ. This polygenetic expression increases the possibilities that an antigen can be presented and recognized by a specific T-lymphocyte. Furthermore, each HLA-gene exists in numerous variations, alleles. In fact, the HLA region is assumed to be the most polymorphic region in the human genome. This polymorphism further contributes to the numbers of possibilities that a certain antigen can be presented in the individual since two alleles of each HLA-gene are inherited, one from each parent, and are co-dominantly expressed. This HLA-polymorphism creates countless of possibilities to combine HLA-molecules and antigens [68, 69].

In contrast to HLA-class I molecules, which are expressed by all nucleated cells, class II expression is restricted to a limited number of cells such as B-lymphocytes, macrophages and dendritic cells. Another important difference is that class I molecules present antigens originating from the intracellular space while antigens presented by class II molecules have an extracellular origin. A consequence is that the origin of the presented antigen will influence the immune response since recognition of HLA-class I molecules are restricted to CD8+ T-lymphocytes while class II molecules are recognized by CD4+ T-lymphocytes [68, 69].

Some HLA alleles are associated with certain autoimmune diseases, an indication of involvement of the immune system in the pathogenesis [147]. One of the first associations recognized was between HLA-B27 and ankylosing spondylitis [15, 125].

Several possible mechanisms for the involvement of certain HLA alleles in the pathogenesis have been suggested [99]. Certain alleles may bind self-antigens too weak, which can result in a failure to eliminate self-reactive T-lymphocytes in the thymus. Another possibility is genetic linkage between the HLA allele and another gene that is involved in the pathogenesis, thus the role for the HLA allele would be to serve as a genetic marker.

As previously mentioned, the HLA region is one of the best-studied candidates for the genetic contribution to sarcoidosis [84]. Associations with HLA-class I and class II alleles have been detected in several studies, both concerning disease susceptibility but also the clinical course [1, 9, 44, 60, 82]. However, the associations vary between different populations, a fact that reflects the heterogeneity of the disease [102]. Methodological differences may also explain some divergent results since older serological methods are less specific than the PCR based methods preferred today [98].

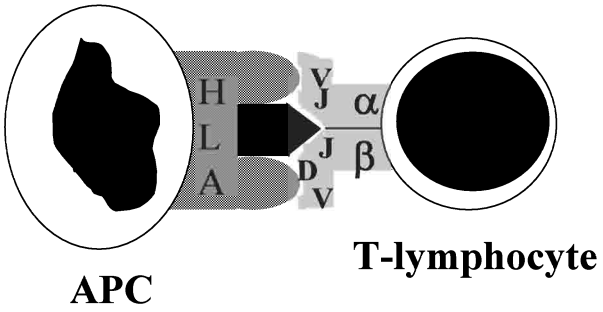

Figure 3. *Schematic outline of the antigen presenting process between an antigen presenting cell (APC) and the T-lymphocyte. Abbreviations*, see **ABBREVIATIONS.**

Regulation of T-lymphocyte activity

The process that leads to the TCR specificity does not *per se* exclude development of self reactive T-lymphocytes and therefore the immune system demands regulatory mechanisms to prevent harmful immune reactions (reviewed in [141]). Potential hazardous T-lymphocytes must be taken care of through mechanisms commonly referred to as central and peripheral tolerance. Central tolerance takes place in the thymus where self-reactive immature lymphocytes are eliminated by apoptosis in a process called negative selection. The subsequent positive selection implies elimination of T-lymphocytes unable to recognize self-HLA molecules. Since some self-reactive T-lymphocytes nevertheless will slip through, additional defence mechanisms are required in the periphery to prevent development of autoimmune diseases [122].

One example of how the immune system has dealt with this issue is the two-signal hypothesis for T-lymphocyte activation. According to this hypothesis T-lymphocytes that recognize antigens without simultaneous co-receptor signals from activated antigen presenting cells (APC) will not be activated and may become functionally inactivated, anergic. A consequence of this mechanism might be inactivation of self-reactive T-lymphocytes since the APC that display the self-antigens are not activated and thus do not supply the co-signal. Another mechanism for induction of peripheral tolerance is through active suppression by regulatory T-lymphocytes (Treg cells). Treg cells have been found to mediate immune tolerance in animal models of autoimmune diseases, graft transplants and infectious diseases [33, 52, 76] and recently lymphocytes with regulatory properties were described in blood samples from healthy humans [6]. Similar to Treg cells obtained in animal models, human Treg cells seem to be of the CD4 subtype, express CD25 and markers associated with memory subsets, CD45RO.

A prominent feature in sarcoidosis is the impairment of delayed-type hypersensitivity against tuberculin and other antigens [43, 133]. Reduction of other T-lymphocyte mediated

functions such as graft rejection and sensitization against contact allergens have also been observed [43]. The underlying mechanisms for this phenomenon remains unclear, one speculation has been that sensitized lymphocytes are redistributed to sites of inflammation from the periphery [43]. Impaired T-lymphocyte function would thus be a reflection of lymphopenia, a condition detected in many sarcoidosis patients [55]. Another explanation could be that cells involved in peripheral tolerance, *e.g.* Treg cells, contribute to the observed impairment. A similar speculation was presented more than 20 years ago, however then based on the assumption that CD8+ cells exert regulatory functions [55].

Resolution of inflammation or development of fibrosis

Why the granulomatous inflammation is resolved in some sarcoidosis patients while maintained and subsequently replaced by fibrosis in other remains a key question [133]. A reasonable hypothesis is that the difference reflects a successful respectively unsuccessful elimination of an eliciting antigen(s) though this does not exclude other mechanisms [89].

Apoptosis, programmed cell death, constitute a physiologic mechanism for maintenance of cellular homeostasis [46]. Our current understanding of this highly conserved machinery originates from studies of cell regulation in the nematode *C. elegans* initiated by Brennan in the 60^{ths} [121]**.** Subsequently the genetic background has been characterized, first in *C. elegans* [28] and then the human homologies. A reflection of the vital importance of apoptosis is the numerous disorders that may evolve from dysregulation [46]. The immune system utilizes programmed cell death for several purposes. Besides elimination of self-reactive T-lymphocytes, death induction of virus infected cells *etc*, apoptosis constitute an important mechanism for resolution of inflammation after successful removal of a hazardous agents [18]. A consequence of dysregulated apoptotic machinery may thus be a prolonged and harmful inflammatory reaction. An example of resistance against apoptotic stimuli is suggested in T-lymphocytes localized at the site of inflammation in Mb Crohn [101], though it remains unclear if this mechanism contributes to the pathogenesis. Another possibility is that lymphocytes become less susceptible to apoptotic stimuli upon antigen stimulation, thereby prolonging their functional lifetime. Recent studies on blood lymphocytes from healthy controls support this assumption since resistance against apoptotic stimulation was seen upon activation [134]. Interestingly, the resistance found was paralleled with increased activity of enzymes that exert central functions in the apoptotic machinery, caspases, thus disclose other cellular functions for these proteases [65]. An altered expression of Fas, FasL and other molecules involved in apoptotic regulation has been detected on cells involved in the inflammation from sarcoidosis patients [2, 71]. Still though the significance of this remain unclear.

Speculations concerning the role for cytokines in development of fibrosis in sarcoidosis have been presented. One hypothesis is that fibrosis is initiated upon a shift from anti-fibrotic Th1 cytokines to more pro-fibrotic Th2 cytokines [2, 89, 133]. Such development has been detected in a *Schistosoma mansoni* model of granulomatous disease [72]. A shift from Th1 to Th2 response could serve as a down-regulating mechanism of a potential hazardous Th1 response, as described in tuberculosis [105]. In a subset of sarcoidosis patients the result may be an over-expression of pro-fibrotic Th2 cytokines instead.

What is so interesting with HLA-DR17 positive sarcoidosis patients?

Ten years ago members of our research group reported that a subgroup of Scandinavian sarcoidosis patients in BALF had a compartmentalized expansion of CD4+ T-lymphocytes expressing the TCR V gene segment Vα2.3 (named AV2S3 today) [38]. Interestingly, these patients were all positive for HLA-DR3 (DR17/DRB1*0301 with present nomenclature), an association recently strengthened in a study including many more subjects [40]. Nucleotide analysis of the α-chain's antigen recognition region, the CDR3 region, revealed differences in nucleotide sequence, thus excluding a monoclonal expansion. However, some of these sequences coded for identical aminoacid sequences [37]. In conclusion, these results implicate that a subgroup of sarcoidosis patients with the capacity to display an antigen in a specific way, by the HLA-DR17 molecule, have an accumulation of key cells that express structural similarities of their antigen recognition receptor, AV2S3. A plausible explanation for this phenomenon is that it reflects a reaction towards a specific antigen. Hitherto this hypothesis has not been refuted though subsequent studies have led to a modified view regarding the role for these cells in the pathogenesis.

Interestingly, DR17 positive sarcoidosis patients also share several clinical features [9]. Since about one third of the sarcoidosis patients visiting our department were positive for this allele, compared to only 17% in healthy controls, DR17 is strongly over-represented among Scandinavian sarcoidosis patients. HLA-DR17 positive patients frequently have an acute disease presentation, often with Löfgren's syndrome, and display less advanced chest x-ray changes compared to DR17 negative patients. Furthermore, DR17 is prognostic favourable since it is associated with a high rate of spontaneously resolving disease within two years from disease onset [9]. The same study revealed further evidence for the prognostic impact of HLA-DR since patients positive for DR14 or 15 more often developed a prolonged, chronic course compared to patients negative for these alleles.

The accumulation of AV2S3+ T-lymphocytes seem to be restricted to sarcoidosis since no clear accumulation have been found in DR17 positive healthy controls or patients suffering from other inflammatory diseases in the lungs [39, 138]. Observations from DR17 positive sarcoidosis patients of non-Scandinavian origin or with a monozygotic twin indicate that the association may outweigh race and is influenced by more than the genetic background [35, 36].

Besides HLA-DR17 positive patients, a corresponding AV2S3+ T-cell expansion has also been detected in DR17 negative sarcoidosis patients positive for the HLA-DR allele DRB3*0101 [40]. Interestingly, DRB1*0301 (DR17) and DRB3*0101 share molecular similarities, especially in regions important for antigen binding [100], thus strengthening that the accumulation is antigen specific.

What role do the AV2S3+ T-lymphocytes play? Since they are associated with a potential harmful inflammatory reaction one may assume that they exert harmful functions. Interestingly though they are only detected in a subgroup of patients that is characterized by a favourable prognosis. Furthermore, the percentage of AV2S3+ CD4+ T-lymphocytes in BALF correlates negatively with disease duration, *i.e.* the more AV2S3+ T-lymphocytes in BALF the shorter disease course, thus indicating protective properties for these cells [34]. It is possible that these cells lead to a more effective eradication of a sarcoidosis specific antigen or impede a harmful inflammatory response by influencing other cells.

A close clinical and immunological characterization of this distinct patient group gives important insights in the inflammatory reaction in sarcoidosis. Such studies could lead to new immunomodulating therapies [127] and may also enable the detection of an eliciting antigen.

AIMS OF THE PRESENT STUDY

The aims of this thesis were to

- characterize the immune response in a distinct group of sarcoidosis patients.
- investigate if a polymorphism in the ACE gene is linked to HLA-DR alleles of prognostic significance.
- analyze apoptotic mechanisms in lymphocytes from sarcoidosis patients.
- investigate if an accumulation of AV2S3+ CD4+ T-lymphocytes in BALF from HLA-DR17 positive patients with active sarcoidosis is normalized after clinical resolution.
- find markers in BALF and peripheral blood representing disease activity in sarcoidosis.

METHODOLOGICAL ASPECTS

A brief description of the methods used in this study is presented below. More detailed descriptions are found in respective original paper.

Study subjects (I-V)

Sarcoidosis patients included in this thesis were of Scandinavian origin, revealed a clinical picture in accordance with sarcoidosis without evidence for any resembling disease. The diagnosis was further based on one or several of the following criteria: a biopsy with non-caseating epithelioid cell granulomas, Löfgren's syndrome, a BALF CD4/CD8 ratio >3.5-4. Disease stage was based on chest radiographics with stage 0 represented a normal chest x-ray, stage I and II represented BHL without or with parenchymal infiltration respectively and stage III revealed parenchymal infiltration without BHL.

Clinically resolved disease was defined as disappearance of symptoms (dry cough, fever, fatigue, arthralgia), a normalized chest x-ray and normal spirometry.

Controls included were all healthy subjects without evidence of pulmonary or other inflammatory disorders.

BAL and handling of BALF (I, III-V)

BAL was performed in local anaesthesia with a flexible bronchoscope wedged in a middle lobe bronchus. BALF was obtained by instillation and gentle aspiration of five 50 ml aliquots of phosphate buffered saline or 0,9% sodium chloride. The recovered BALF was strained through a Dacron net, centrifuged once and subsequently separated into supernatant and cell pellet, respectively. Cellular concentration and viability of cells were counted in a Bürker chamber after staining with trypan blue. Cell differentiation was counted on cytospin preparations after staining with May-Grünwald and Giemsa solutions.

Separation of peripheral blood mononuclear cells (PBMC) (III-V)

PBMC were separated from blood samples by Ficoll-Hypaque gradient centrifugation.

Soluble components in serum and BALF (I, II, IV, V)

Analyses of neopterin and sIL2R in serum and fibronectin in BALF were performed with ELISA technique. BALF levels of albumin and procollagen III N-terminal peptide were measured by nephelometry and radioimmunoassay respectively while SACE was determined by methods based on either spectrophotometry (I) or colorimetry (II, IV, V).

Detection of surface molecules on lymphocytes (I, IV, V)

Surface molecules expressed by lymphocytes were detected after staining with mAb followed by analysis in a flow cytometer. Lymphocytes were recognized and gated by light scattering properties. Isotyped matched mAb served as negative controls and stained less than 1% of lymphocytes.

Analysis of apoptotic phenotype and caspase-3 activity (III)

Two methods were used to identify apoptotic lymphocytes. Through identifying nuclear condensation by staining with Hoechst 3342 and by flow cytometry detection of

phosphatidylserine with annexin V. The caspase activity was determined by usage of a cell permeable caspase-3 substrate, PhiPhilux-G2D2, by flow cytometry.

Typing of HLA-class II (I, II, IV, V) and ACE insertion/deletion alleles (II)

DNA was extracted from blood leukocytes for analysis of HLA-DR alleles in patients and of the ACE I/D allele in patients and controls. PCR techniques were then applied for the determination of respective alleles.

RESULTS AND DISCUSSION

Paper I

Results

BALF from 67 DR17 positive and 51 DR17 negative sarcoidosis patients were analyzed for cellular concentration, cellular profile and the CD4/CD8 ratio. Compared to DR17 negative patients, BALF from DR17 positive patients were characterized by a decreased percentage, and concentration, of lymphocytes and eosinophils but an increased CD4/CD8 ratio.

Analyses of serum between the two groups revealed a decreased ACE activity in DR17 positive patients, a difference that remained when the comparison was restricted to patients with stage I disease. Furthermore, serum levels of neopterin and sIL2R were analyzed in subgroups of respective patientgroup and 10 healthy controls. Levels of both neopterin and sIL2R were lower in DR17 positive patients compared to DR17 negative. In their turn DR17 positive patients revealed increased neopterin levels compared to controls however no corresponding difference was seen between these groups regarding sIL2R.

What mechanisms underlie the clinical features of DR17 positive sarcoidosis patients?

Several attempts have been made to find out if BALF parameters can be used for prognostic purposes in sarcoidosis [66, 75, 142, 144]. The predictive role for the percentage of lymphocytes has been disputed. Although the prognosis *per se* was not investigated in the present study our results with a relative low percentage of lymphocytes in DR17 positive patients, a group characterized by a good prognosis, contradicts that an increased lymphocytosis predicts a favourable outcome. With a corresponding reasoning concerning the lymphocyte subsets our result supports that an increased CD4/CD8 ratio predicts a favourable outcome, a sign less disputed than the BALF lymphocytosis [23, 142, 144].

Regarding serum parameters, a decrease in SACE found in DR17 positive patients probably reflects a less extensive disease in these patients [32, 94]. Interestingly though, the difference remained when the comparison was restricted to stage I patients.

Neopterin levels are reported to reflect INF-γ activity [54]. Lower levels of neopterin, as in DR17 positive, thus could reflect less activity of this Th1 cytokine. In a recent study lymphocytes obtained from DR17 positive patients expressed a less pronounced intracellular Th1 cytokine profile than DR17 negative patients [139]. One may therefore speculate if the decreased neopterin levels reflect a less pronounced Th1 response in DR17 positive patients compared to DR17 negative patients. Finally, although sIL2R was decreased in DR17 positive patients our data do not support the usage of this marker for estimating disease activity or prognosis in sarcoidosis since it did not differ between this patient group and controls.

Paper II

Results

Investigation of an I/D polymorphism in the ACE gene in 73 sarcoidosis patients and 65 controls did not disclose any difference either for the relative frequency of respective alleles (I and D) or the three resulting genotypes (II, ID and DD) between patients and controls. To detect a possible linkage between this polymorphism and HLA-DR alleles previously found to be of prognostic relevance in Scandinavian sarcoidosis patients, HLA-DR17 and DR14 or 15,

we compared the ACE genotype between patients positive for these HLA-DR alleles. Some minor, though not significant, differences in ACE genotype frequencies were observed.

Sixty-one patients were followed for at least two years and were then classified as non-chronic or chronic due to lack of remaining signs of disease. No difference in ACE genotypes was found between the groups. However, a corresponding comparison for HLA-DR alleles revealed that in 17 out of 19 DR17 positive patients but only in 7 out of 26 DR14 or 15 positive patients the disease had resolved within two years. Finally, we studied the impact of the respective ACE genotype on SACE. A tendency towards increased SACE in DD positive patients compared to II and ID positive patients was detected, though the ACE genotype did not alter SACE significantly in patients. In contrast, SACE in controls differed significantly between subjects positive for the respective ACE genotype, with SACE increasing from II to DD positive individuals with medium activity found in those positive for ID.

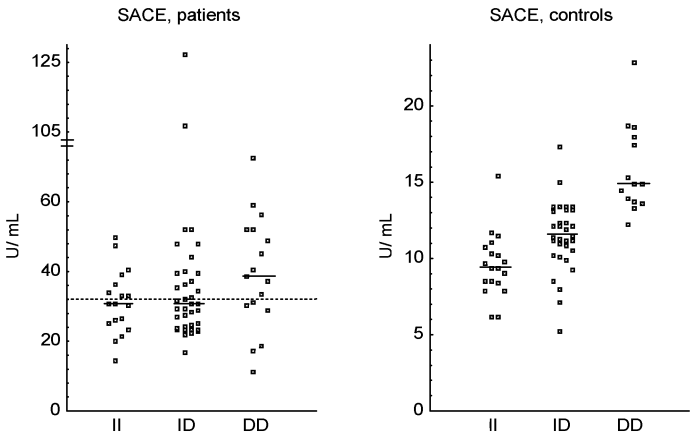

Figure 4. *Serum activity of ACE in relation to ACE genotypes in patients, the upper reference value is marked (dotted line), and controls. Median values are marked as horizontal bars.*

Does the ACE I/D polymorphism affect the clinical course in sarcoidosis?

Some authors have reported that the I/D ACE polymorphism affects the risk of getting sarcoidosis as well as the clinical course of the disease [31, 80, 109]. However, contradicting results exist which may be explained by differences in study populations, for example the ACE genotype has never been found to affect the susceptibility to sarcoidosis in Caucasians [31, 80, 83, 109]. Although relative few subjects were included in the present study, the population was apparently big enough to confirm the prognostic impact of the investigated HLA-DR alleles. A conclusion is therefore that even if the ACE genotype affects the susceptibility for sarcoidosis and/or the clinical course in Scandinavians the impact is far less than the impact of the investigated HLA-DR alleles.

The study confirms the importance of the investigated I/D polymorphism on SACE in healthy subjects, a phenomenon that seems to exist irrespective of ethnical background [5, 31, 117]. A consequence of this observation, discussed in [129, 137], is the increase in sensitivity for SACE if the individual ACE genotype is analyzed and taken into consideration. Compared to healthy controls the I/D polymorphism did not exert any significant influence on SACE in the patients, indicating other mechanisms involved in directing the ACE production in sarcoidosis.

Paper III

Results

The ability to induce apoptosis in BALF lymphocytes and peripheral blood lymphocytes (PBL) from sarcoidosis patients and healthy controls was tested. Besides apoptotic phenotype, lymphocytes were also analyzed for the activity of a central enzyme in the apoptotic machinery, caspase-3. Percentage of apoptotic phenotype was approximately equally low in unstimulated BALF and blood lymphocytes, and did not differ between patients and healthy controls. When apoptotic stimuli were added, a clear increase in the percentage of apoptotic lymphocytes was detected in BALF from healthy controls and PBL from both healthy controls and sarcoidosis patients though not in BALF lymphocytes from sarcoidosis patients. A similar pattern was found when caspase-3 activity was analyzed. Unstimulated BALF lymphocytes from healthy controls revealed increased caspase activity compared to the activity detected in PBL from both patients and controls. The highest activity though was found in BALF lymphocytes from sarcoidosis patients. Upon apoptotic stimulation caspase-3 activity was increased in BALF lymphocytes from controls and PBL from both controls and patients. In contrast, apoptotic stimulation did not alter the caspase-3 activity in BALF lymphocytes from sarcoidosis patients.

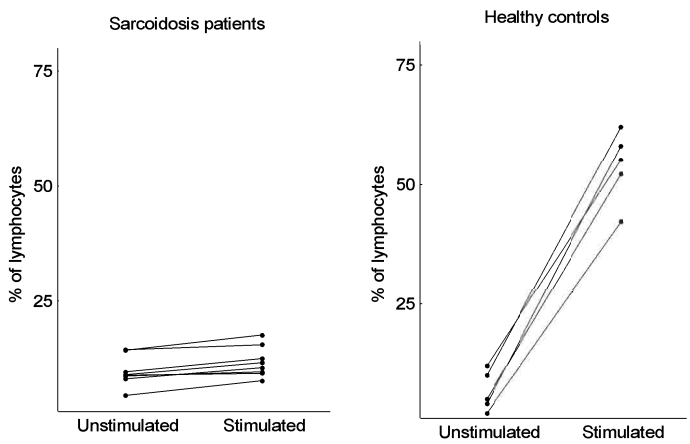

Figure 5. *Percentage of lymphocytes in BALF from sarcoidosis patients and healthy controls exhibiting apoptotic phenotype before and after stimulation with apoptotic stimuli.*

Is a reduced susceptibility to apoptosis harmful?

Findings in patients suffering from inflammatory bowel disease suggested a reduced apoptotic capacity in T-lymphocytes accumulated at the site of the inflammation [101]. A speculation is that such decrease in apoptotic capacity is harmful and leads to a prolonged inflammatory reaction. However, a reduction in apoptotic capacity may also be physiologic relevant, preventing a premature death of immune cells and thereby improve the response [134]. Recently, caspase-3 activity in T-cells has been reported to be increased upon T-lymphocyte activation without evidence for ongoing apoptosis [65]. In a study of PBL from healthy donors, caspase-3 activity was sustained for 3-5 days after T-cell stimulation [134]. Similar to our results, a resistance against apoptosis paralleled the increased caspase activity. Thus, it is unclear if our finding with a reduced ability to induce apoptosis in BALF lymphocytes reflects a physiologic or a pathologic mechanism in sarcoidosis.

Paper IV

Results

BALF and serum were obtained from 9 DR17 positive sarcoidosis patients at disease onset and after clinical and radiological resolution. Analyses revealed that a restricted accumulation of AV2S3+ CD4+ T-lymphocytes, present in BALF at disease onset, was normalised at follow-up in all patients. Furthermore, a significant decrease in BALF lymphocytes and strong tendencies towards decreased cellular concentrations and CD4/CD8 ratios in BALF were detected at the follow-up examination. In serum, ACE activity was declined in all included patients at clinical resolution though it was only exceeding normal ranges in three patients at disease onset. A reduction was also detected in serum levels of neopterin in 8 of 9 patients from disease onset to clinical resolution.

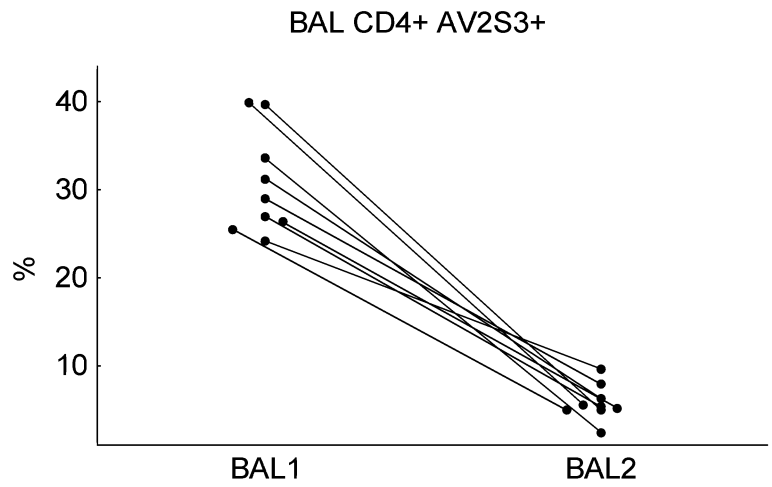

Figure 6. *Percentage of CD4+ BALF cells expressing AV2S3 at disease onset (BAL1) and after clinical resolution (BAL2).*

Does clinical resolution of sarcoidosis reflect removal of a specific antigen?

The results obtained in this study confirm that AV2S3+ CD4+ T-lymphocytes are involved in the inflammatory reaction in DR17 positive sarcoidosis patients. Somewhat unexpected, although the percentage of BALF AV2S3+ cells was normalized in all patients at clinical resolution, elevated levels of the lymphocyte percentage and the CD4/CD8 ratio remained in one respectively two patients. These three patients fulfilled the criteria for clinical resolution but they had in common a relative short interval between the two BAL investigations, just around two years. This finding may suggest that normalization in the percentage of AV2S3+ CD4+ T-lymphocytes in BALF precedes normalization of BALF lymphocytes and CD4/CD8 ratio.

SACE was decreased at clinical resolution in all included patients which supports that it reflects disease activity. However, the clinical value of this observation is limited by the fact that SACE in the majority of patients were within normal ranges at disease onset. The reduction in serum neopterin from disease onset to clinical resolution suggests that it is reflecting inflammatory mechanisms involved in sarcoidosis. Similar to SACE though its usage as a marker for disease activity may be restricted by the observed overlap between disease onset and clinical resolution in a few patients.

Paper V

Results

The CD4+ lymphocyte expression of activity markers CD69, HLA-DR and CD25 in BALF and peripheral blood was analyzed in 23 DR17 positive patients with active sarcoidosis, 9 DR17 positive patients with resolved disease and 36 healthy controls.

A clear decrease in the percentage of HLA-DR+ CD4+ T-lymphocytes in BALF from resolved patients and controls compared to patients with active disease was found. A less, though still significant, decrease in CD25+ CD4+ BALF T-lymphocytes was seen in patients with resolved compared to active disease. However, a wide range of the percentage of CD4+ BALF T-lymphocytes expressing CD25 was detected among patients with active disease.

A similar pattern was seen in peripheral blood lymphocytes with a significantly decreased expression of HLA-DR and CD25 on CD4+ T-lymphocytes from patients with resolved disease and healthy controls compared to patients with active disease. However, in contrast to the results in BALF, differences between patients with resolved and those with active disease were less clear-cut regarding HLA-DR+ but far more pronounced for the CD25 expression. Interestingly, a negative correlation was found in patients with active disease between the percentage of CD25+ CD4+ BALF T-lymphocytes and cellular markers of alveolitis, most pronounced for BALF concentration of lymphocytes.

No differences were detected for the percentage of CD69+ CD4+ lymphocytes either in BALF or peripheral blood between active and patients in remission or between any patient group and controls. However, within each group a remarkable difference in CD69 expression was detected between BALF and peripheral blood.

Does the expression of activity markers on CD4+T-cells reflect disease activity?

This study revealed that clinical resolution of sarcoidosis in DR17 positive patients is associated with a decreased expression of HLA-DR and CD25, but not CD69, on CD4+ T-lymphocytes. A variation of CD25+ expression in BALF lymphocytes from sarcoidosis patients has previously been reported [97]. Several explanations for this phenomenon have

been suggested, such as shedding of the CD25 molecule or trafficing of CD25+ cells to other compartments [95]. Another possibility could be that the CD25+ CD4+ T-lymphocyte population consists of different functional subsets. Lymphocytes exerting regulatory properties on the immune response, Treg cells, have been found to express CD4, CD25 and CD45RO [6]. Since more than 90% of BALF lymphocytes express CD45RO [64], we speculate that Treg cells contribute to the CD25+ CD4+ T-lymphocyte populations in patients with active sarcoidosis. Such contribution may explain the inverse correlation between the percentage of CD25+ CD4+ T-lymphocytes in BALF and cellular signs of alveolitis in this study but also the impairment of delayed hypersensitivity that is common in sarcoidosis [43, 133].

Irrespective of their functional role, the differences in HLA-DR and CD25 expression on CD4+ lymphocytes between patients with resolved sarcoidosis and those with active disease imply that they could be useful as markers for disease activity in this patient population.

CONCLUDING REMARKS

The present study showed that BALF from HLA-DR17 positive patients is characterized by an increased CD4/CD8 ratio but a decreased lymphocytic percentage compared to DR17 negative patients. SACE as well as serum levels of neopterin and sIL2R were also lower in DR17 positive patients.

No association between the investigated ACE polymorphism and susceptibility to sarcoidosis was found. Neither was there any link between this ACE polymorphism and certain HLA alleles which are of prognostic significance in Scandinavian sarcoidosis patients, *i.e.* HLA-DR17, DR14 and DR15. The study did not reveal any prognostic impact of the investigated ACE polymorphism, however the results confirmed the previously reported role for HLA-DR as a prognostic marker. The ACE genotype in patients tended to influence SACE while a significant association was seen between SACE and ACE genotype in healthy individuals.

BALF lymphocytes, but not PBL, from sarcoidosis patients were resistant to induction of apoptosis. This resistance was paralleled by an increased caspase-3 activity which was unaffected by apoptotic stimulation. In contrast, neither BALF lymphocytes from healthy controls nor PBL from patients or controls were resistant to apoptosis. In addition, caspase-3 activity in lymphocytes from these three populations was induced by apoptotic stimulation.

A significant association was demonstrated between clinical resolution of sarcoidosis and a normalization of AV2S3+ CD4+ T-lymphocytes in BALF in HLA-DR17 positive patients. This normalization was associated with remission of other signs of disease activity as decreased BALF cellular concentration, lymphocyte percentage, CD4/CD8 ratio, SACE and serum neopterin levels.

The expression of HLA-DR and CD25, but not CD69, on BALF and blood CD4+ T-lymphocytes was significantly lower in clinically resolved DR17 positive sarcoidosis patients compared to DR17 positive patients with active disease.

Future aspects

The results strengthen our hypothesis that AV2S3+ CD4+ T-lymphocytes are involved in the pathogenesis of sarcoidosis in HLA-DR17 positive patients. A closer characterization of their role in the inflammatory reaction would therefore increase our understanding of the mechanisms involved in this puzzling disease. In particular, by testing the AV2S3+ T-lymphocyte reactivity towards candidate antigens, a sarcoidosis associated agent could eventually be identified. Another interesting future aspect would be to learn what factors influence the progression or resolution of inflammation in DR17 negative versus DR17 positive patients. The mechanisms behind the apoptotic resistance in sarcoidosis BALF T lymphocytes would be important to understand, as well as the significance of this apoptotic phenotype for the development of chronic inflammation. Finally, the role for T regulatory lymphocytes in BALF in both health and disease deserves further attention.

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