Johan Grunewald

Identification of antigens in BAL fluid in Sarcoidosis

Abstract

Activated T lymphocytes of the Th-1 type are typically accumulated in the lungs of patients with sarcoidosis, and there they may recognize specific sarcoidosis antigens, presented by antigen presenting cells such as dendritic cells and/or alveolar macrophages. In line with this, previous studies have indicated alveolar macrophages to contain proteins that could induce granuloma formation. Also, signs of a specific immune response in the lungs of HLA--DRB1*0301 positive sarcoidosis patients suggest the presentation of specific antigens by HLA class II molecules on BAL cells. A number of peptides have therefore been eluted from HLA-DR molecules of BAL cells of HLA-DRB1*0301 positive sarcoidosis patients and these peptides are now used in stimulation tests to analyse their capacity to elicit T cell responses, using an elispot assay. Interestingly, all peptides identified so far were derived from self proteins, indicating autoimmune reactions in the lungs of these patients. These studies may lead to the identification of (a) sarcoidosis specific antigen(s).

Key-words: BAL fluid; proteomics; sarcoidosis antigens

Sarcoidosis is a granulomatous disease of unknown etiology, commonly affecting the lungs. Although most patients recover, some develop chronic disease with in some patients fibrosis and respiratory failure[1]. Activated CD4+ T helper cells accumulate in the lungs of sarcoidosis patients and there they produce large amounts of Th1 associated cytokines (Fig. 1)[2-4]. A restricted T cell receptor (TCR) repertoire of the bronchoalveolar lavage (BAL) fluid T cells, with a preferential expression of certain TCR α or Vβ genes, has been reported in several studies, suggesting T cell reactivity against spe-

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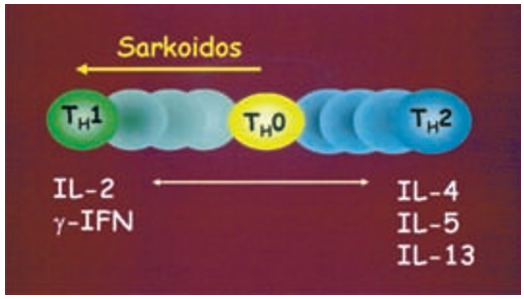

Fig. 1 – Lung accumulated T cells typically produce Th-1 associated cytokines.

cific sarcoidosis-associated antigens in the lungs[5-9]. Especially the strong correlation between DRB1*0301 and dramatic expansions of oligoclonal TCR AV2S3+ CD4+ T cells in the BAL fluid of sarcoidosis patients[10] strongly indicate that they have been interacting with specific antigens[6,11]. Altogether, these findings suggest the presence of sarcoidosis specific antigens in the lungs of the patients (Fig 2).

Several studies by Drs Y Kataria and J Holter have indicated the presence of a factor, called a granulomagenic factor, capable of inducing granulomas in sarcoidosis patients[12]. By injecting non-viable autologous BAL cells (NVBC) into the skin of sarcoidosis patients, and at the same time injecting Kveim-Silzbach

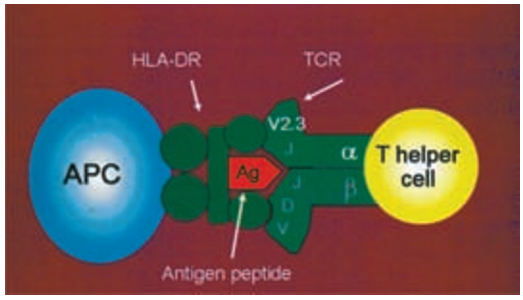

Fig. 2 – Specific antigen presentation by HLA-DRB1*0301 molecules and antigen recognition by T cells expressing the TCR AV2S3 gene segment.

antigens at other sites, these authors found that in about half of the patients, granulomas appeared following injections with the NVBC. Patients with a positive response were those with a recent onset of disease, and the granulomas were indistinguishable from granulomas resulting from Kveim-Silzbach antigen injections. Subsequent studies indicated that the granulomagenic factor was derived from alveolar macrophages (AM) rather than BAL lymphocytes, and the authors could also report granuloma formation following injections with membrane fractions of AM. The granulomagenic factor in these studies hás been suggested to include sarcoidosis specific antigens, presented to T cells by AM. For future studies, intracellular and/or membrane associated proteins in certain BAL cell subsets such as the alveolar macrophages should therefore be interesting to study. Such analyses may reveal a distinct protein production and presentation by the cells, which might relate to the specific inflammatory process seen in sarcoidosis.

In our search for sarcoidosis-specific antigens, we set out to identify peptides presented by HLA-DR molecules of BAL cells of patients with sarcoidosis. Since we previously found signs of a specific immune response in HLA--DRB1*0301 positive patients, we focused on this particular subset of patients, and pooled BAL cells of 15 DRB1*0301 positive sarcoidosis patients. In collaboration with Dr. R Weissert and Dr. H-G Rammensee at the University of Tübingen, Germany, HLA class ll-bound peptides were isolated according to standard protocols, using an HLA-DR specific antibody, acid treatment, ultrafiltration and fractionation by HPLC (Fig. 3). Peptide--containing HPLC fractions were pooled and aliquots analyzed by nanocapillary HPLC elec-

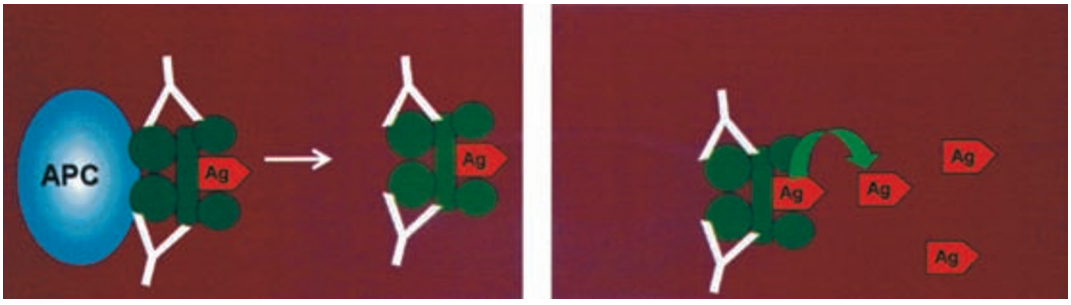

Fig. 3 – HLA-DR molecules were separated, using HLA-DR specific antibodies, and antigen peptides were eluted, according to Standard protocols. Peptides were then separated and sequenced.

trospray ionization (HPLC ES) MS. Peptide mass fingerprints were analyzed manually for identification of a tentative amino acid sequence. We have identified 87 definite amino acid sequences derived from 38 different human proteins as defined by data base searches performed in collaboration with Dr B Persson at the Centre for Genomics & Bioinformatics, Kl. All the identified proteins are self-proteins, involved in antigen processing and presentation, from cell surface receptors, cytoskeletal proteins, enzymes, housekeeping proteins, serum proteins or various (e.g. surfactant) proteins.

Our data on peptides identified from BAL cells of DRB1*0301 positive sarcoidosis patients is for the first time showing what antigens are locally presented during a pulmonary inflammatory condition. Several of these were normally abundant proteins such as albumin. Other proteins, such as some intracellular enzymes, could better be anticipated to serve as targets for autoimmune attacks and in fact one of these proteins is in a HLA-DRB1*0301-associated way involved in pulmonary inflammation connected to another autoimmune disease. We now use these candidate antigens in stimulation tests to elucidate their role in the pathogenesis of sarcoidosis. A few of the tested peptides are in fact capable of stimulating T cells of sarcoidosis patients, but not controls, to produce IFNy. Although laborious, these studies may lead to the identification of sarcoidosis-specific antigen(s).

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