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Review

Humoral immune profiling of mycobacterial antigen recognition in sarcoidosis and Löfgren's syndrome using high-content peptide microarrays

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SUMMARY

Introduction: Sarcoidosis is considered an idiopathic granulomatous disease, although similar immunological and clinical features with tuberculosis (TB) suggest mycobacterial involvement in its pathogenesis. High-content peptide microarrays (HCPM) may help to decipher mycobacteria-specific antibody reactivity in sarcoidosis.
Methods: Serum samples from patients with sarcoidosis, Löfgren's syndrome, and TB, as well as from healthy individuals (12/group), were tested on HCPM containing 5964 individual peptides spanning 154 *Mycobacterium tuberculosis* proteins displayed as 15-amino acid stretches. Inclusion/exclusion and significance analyses were performed according to published methods.
Results: Each study group recognized 68–78% *M. tuberculosis* peptides at least once. *M. tuberculosis* epitope recognition by sarcoidosis patient sera was 42.7%, and by TB patient sera was 39.1%. Seven and 16 peptides were recognized in 9/12 (75%) and 8/12 (67%) sarcoidosis patient sera but not in TB patient sera, respectively. Nine (75%) and eight (67%) out of twelve TB patient sera, respectively recognized *M. tuberculosis* peptides that were not recognized in sarcoidosis patient sera.
Conclusions: Specific IgG recognition patterns for *M. tuberculosis* antigens in sarcoidosis patients re-affirm mycobacterial involvement in sarcoidosis, providing biologically relevant targets for future studies pertaining to diagnostics and immunotherapy.
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Introduction

Sarcoidosis is a disorder of unknown aetiology, characterized by non-caseating granulomatous inflammation sustained by CD4 T-cell activation. It affects mainly lung and hilar lymph nodes, but has a propensity to affect every organ/tissue.[1] The disease is characterized by a wide spectrum of clinical manifestations, from the acute, self-limiting Löfgren's syndrome, to chronic fibrotic forms, suggesting a multifactorial pathogenesis. The correlation with specific major histocompatibility complex (MHC) class II molecules, in addition to recruitment and expansion of specific subsets of CD4 T-cells in the alveolar space in well-defined patient subgroups (i.e., Löfgren's syndrome), suggests that antigen-driven inflammation plays a critical role.[2–4]

Since sarcoidosis and pulmonary tuberculosis (TB) are both granulomatous diseases, and since they share a similar distribution of the lesions in the lung, mycobacterial species have been incriminated in the pathogenesis of sarcoidosis.[1,5,6] *Mycobacterium tuberculosis*-derived antigens and DNA have been isolated from lung granulomas,[1] while T-cell reactivity to several mycobacterial proteins has been demonstrated in subgroups of patients with sarcoidosis.[1,7–11] However, since the specificity and reproducibility of these observations are limited, the CD4/CD8 ratio in bronchoalveolar lavage fluid (BALF) remains the diagnostic benchmark. While patients with Löfgren's syndrome may clear mycobacterial antigens from the systemic circulation to resolve granulomatous inflammation, individuals with chronic fibrotic forms of systemic sarcoidosis may not be able to do so, succumbing to end-organ damage and fibrosis.[2]

Immunoglobulin G (IgG) molecules are increased in the blood and BALF of sarcoidosis patients, suggesting a potential role for antigen-specific humoral immune responses in triggering sarcoid inflammation.[1] The discovery that serum IgG from subgroups of patients with chronic sarcoidosis binds to *M. tuberculosis* proteins (catalase, purified protein derivatives) in the Kveim reagent (used for diagnosis), suggests mycobacterial involvement in granulomatous inflammation in sarcoidosis.[12,13] Nevertheless, due to technical constraints, it has not been possible to gauge the entire spectrum of antibody responses to mycobacterial antigens.

High-content peptide microarrays (HCPM) now offer the opportunity to test the presence of immunologically relevant epitopes with small quantities of clinical material.[14] Using this technique, the presence of IgG molecules specific for a high number of epitopes derived from human pathogens may be visualized and quantified, as has been reported previously for TB, influenza, cytomegalovirus infection, and pertussis.[15–19] This information can then be processed using statistical methods in order to generate an immune recognition landscape for each protein, based on the IgG recognition of individual epitopes.

This study using an HCPM platform customized with 154 *M. tuberculosis* antigens was performed to describe the differential humoral immune responses to a high number of *M. tuberculosis* antigens in serum from patients with Löfgren's syndrome, sarcoidosis, and active pulmonary TB, as well as in serum from healthy individuals, by means of qualitative and quantitative analyses. Individual *M. tuberculosis* epitopes with potential implications in the differential diagnosis of sarcoidosis, Löfgren's syndrome, and pulmonary TB are discussed.

Methods

Design

The study was designed as a cross-sectional comparison of 'reactosomes' (i.e., the overall immune profile detected on the peptide microarray, composed of the mean intensity of recognition and the number of recognitions over the specific peptides) in four groups of subjects: healthy controls (HTC), patients with active TB (TB), patients with sarcoidosis (SARC), and patients with Löfgren's syndrome (LOF). Every group comprised 12 serum samples, each from a single volunteer, matched by ethnic group (all the enrolled subjects were Swedish), age, and sex, in order to avoid recruitment biases in the data analysis. However, sex matching was not entirely possible for the Löfgren's syndrome and sarcoidosis groups.

Serum samples

Sera from 12 healthy subjects were obtained from collections from the Lung Research Laboratory and from the Department of Microbiology, Tumour and Cell Biology, Karolinska Institutet, Stockholm, Sweden. Sera from patients with sarcoidosis and Löfgren's syndrome were obtained from a collection of the Lung Research Laboratory, Karolinska Institutet, Stockholm, Sweden. Sera from patients with active TB were obtained from the Sahlgrenska Hospital, University of Goteborg, Goteborg, Sweden, and from the Clinic of Infectious Diseases, Huddinge University Hospital, Karolinska Institutet, Stockholm, Sweden. All samples were collected and used after the necessary institutional review board approval had been obtained (2005/1031-31 and 2009/20-32, approved by the Regional Ethics Review Board in Stockholm). All subjects who actually donated blood for the study signed an informed consent form.

Microarray slides and experiments

Peptide microarray slides were customized and manufactured by JPT (Berlin, Germany).[20] The slides contain three identical sub-arrays with 5964 unique peptides on each sub-array. Each sub-array contains 16 blocks arranged in a regular pattern, with spots arranged in a 16×15 matrix (a schematic representation of a microarray and a table containing a list of the proteins displayed on the chip are available in the **Supplementary Material** in the online version at doi: http://dx.doi.org/10.1016/j.ijid.2017.01.021; Figure S1, Table S1). Each sub-array contains positive controls, negative controls, the unique peptides spanning 154 *M. tuberculosis* proteins of interest (Table S1), totalling 17 892 spots per slide. The entire amino acid sequence of each *M. tuberculosis* protein was printed on the microarray as 15-mer amino acid peptides overlapping the

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