

INSIGHTS

# A fungal antigenic driver for Löfgren's syndrome sarcoidosis

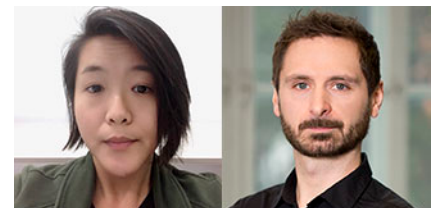
Clarice X. Lim and Thomas Weichhart

**Löfgren's syndrome is an acute form of sarcoidosis that is characterized by the activation of CD4<sup>+</sup> T helper cells. In this issue of JEM, Greaves et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20210785>) identified a peptide derived from an airborne mold species that stimulates T cells of Löfgren's syndrome patients in an HLA-DR3-restricted manner. An increased serum IgG antibody response to the full-length protein was also observed in those patients, indicating that the fungus *Aspergillus nidulans* might be the elusive microbial agent that drives acute sarcoidosis.**

Sarcoidosis is a complex multi-system inflammatory disease of unknown cause, characterized by the presence of non-caseating granulomas in affected organs, primarily the lungs (Valeyre et al., 2014). It clinically presents in two main forms: as an acute form, known as Löfgren's syndrome (LS), and a non-LS form of sarcoidosis that is progressive and nonresolving, even with corticosteroid treatment. LS is characterized by a rapid onset of defined symptoms, such as fever, bilateral hilar lymphadenopathy, erythema nodosum, and ankle swelling, that generally resolves spontaneously over time, and is linked to the carriage of certain HLA alleles (Grunewald and Eklund, 2009). The genetic susceptibility of LS patients carrying the *HLA-DRB1\*03* allele or the *HLA-DRB3\*01:01* allele, MHC class II molecules with similar antigen binding regions, is associated with oligoclonal expansion of specific CD4<sup>+</sup> TCR clones. These express the TCR  $\alpha$  variable 12-1 (*TRAV12-1*) in the lungs of patients with active disease and disappear upon disease resolution. *TRAV12-1* pairs preferentially with *TRBV2* (Mitchell et al., 2017), and the accumulation of clonal populations of *TRAV12-1/TRBV2*-expressing CD4<sup>+</sup> T cells in the lungs of pulmonary sarcoidosis patients carrying the *HLA-DRB1\*03* allele suggest the existence of specific antigens that are presented by APCs to the T cells (Grunewald et al., 2016).

Despite efforts to understand the disease, the causative agents in sarcoidosis remain unknown. Correlative links have been drawn to microbes such as *Mycobacterium* and *Cutibacterium* species, which are detected at higher frequencies in sarcoidosis patient samples compared with healthy controls, and environmental antigens such as silica or metals (Moller et al., 2017). In addition, peptides from the mycobacterial proteins ESAT-6 and katG were previously shown to induce a T helper cell type 1 CD4<sup>+</sup> T cell response in patients expressing the *HLA-DRB3\*01:01* allele (Oswald-Richter et al., 2010). Endogenous molecules such as serum amyloid protein that localize to macrophages and giant cells in sarcoid granulomas or vimentin have also been proposed as disease triggers (Moller et al., 2017). Vimentin was isolated from HLA-DR molecules on sarcoidosis alveolar macrophages and molecularly modeled to be an ideal fit for the peptide-binding groove of the *TRAV12-1/TRBV2*-*HLA-DRB1\*03* binding complex (Kaiser et al., 2019).

In this issue, Greaves et al. (2021) provide compelling evidence that LS patients generate an antigen-specific immune response against a peptide derived from the NAD-dependent protein deacetylase hst4 (NDPD) of the filamentous fungus *Aspergillus nidulans*. Through the use of TCR sequencing, selection of TCRs that mainly express *TRAV12-1* and *TRBV2*, positional



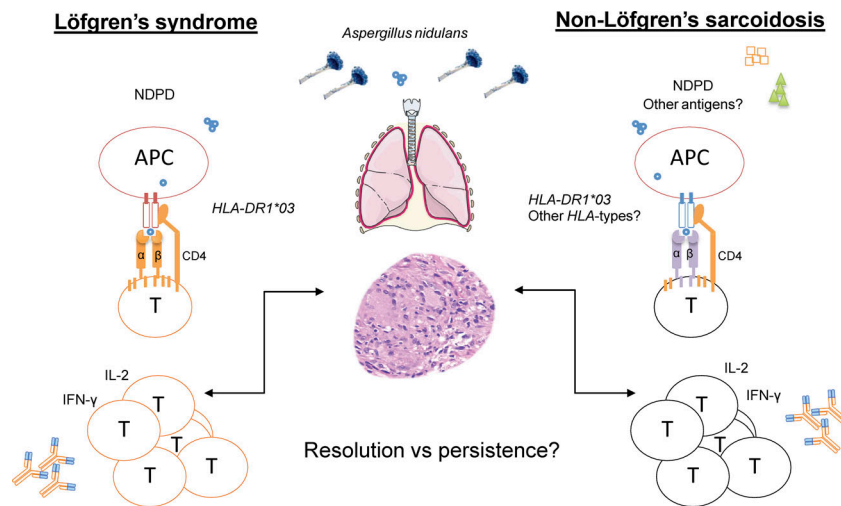
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scanning libraries, and structural and bioinformatic analyses to screen for peptides that bind those disease-relevant TCRs from HLA-DR3<sup>+</sup> LS patients, the authors identified in an unbiased tour de force manner a 10-mer epitope of NDPD from this ubiquitous airborne mold that induced secretion of IFN- $\gamma$  and IL-2 from bronchoalveolar lavage cells in an HLA-DR3-restricted and sarcoidosis-specific manner. Moreover, NDPD was processed by HLA-DR3-expressing fibroblasts to stimulate LS-specific T cell activation. Injection of the *A. nidulans* NDPD peptide into MHC class II-deficient HLA-DR3 transgenic mice generated a specific CD4<sup>+</sup> T cell response against the NDPD peptide in an HLA-DR3-restricted manner. Additionally, they also found increased serum IgG antibody responses to the proposed antigen in LS sarcoidosis patients compared with healthy controls, suggesting past exposure to the

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NDPD derived from the airborne fungi species *A. nidulans* was identified from epitope mapping of TCRs of LS sarcoidosis patients carrying the *HLA-DRB1\*03* allele. HLA-DR3-restricted antigen-specific CD4<sup>+</sup> T cell-derived IL-2 and IFN- $\gamma$ , as well as increased serum IgG, was observed in LS patients. To a lesser extent, the identified NDPD peptide is also recognized by HLA-DR3<sup>+</sup> non-LS sarcoidosis lung cells. The interplay of NDPD and other peptides together with certain HLA haplotypes carried by APCs and the resultant antigen-specific immune responses will likely play an important role in the resolution or persistence of granulomas and clinical severity during LS and non-LS sarcoidosis.

antigen, and a direct causative effect on disease pathogenesis.

Greaves et al. also found that bronchoalveolar cells of *HLA-DR3*-expressing non-LS sarcoidosis patients also react significantly to the identified peptide. In addition, a higher frequency of *TRAV12-1/TRBV2* expressing CD4<sup>+</sup> T cells accumulate in the lungs of *HLA-DR3*<sup>+</sup> patients compared with *HLA-DR3*<sup>-</sup> non-LS patients (Grunewald et al., 2016). Hence, one can speculate that the identified NDPD peptide is involved in disease pathogenesis in both LS and non-LS *HLA-DR3*-expressing sarcoidosis patients. Therefore, it will be interesting to compare the NDPD peptide serum IgG antibody levels between the different LS and non-LS *HLA-DR3* haplotypes. As the expansion of *TRAV12-1* expressing CD4<sup>+</sup> T cells and carriage of *HLA-DRB1\*03* or *HLA-DRB3\*01:01* allele is associated with good clinical prognosis and disease resolution within 2 yr (Darlington et al., 2020; Grunewald and Eklund, 2009), future studies analyzing the disease course of non-LS *HLA-DR3*<sup>+</sup> versus non-LS *HLA-DR3*<sup>-</sup> sarcoidosis patients may reveal if the development of T cell-mediated antigen-specific responses in sarcoidosis perpetuate inflammation, like in berylliosis (Falta et al., 2021), and are associated with disease severity, or if

the development of such antigen-specific immune responses is associated with disease resolution. It would also be interesting to compare the expression of activation/inhibitory receptors on antigen-specific CD4<sup>+</sup> T cells in such a study cohort, as an effector T cell profile was identified to be linked to non-LS sarcoidosis disease progression (Kaiser et al., 2017).

While many studies in sarcoidosis focus on T cells, macrophages (another key player in granuloma formation) and their precursors also display dysregulated processes associated with disease progression (Lepzien et al., 2021; Linke et al., 2017). In that regard, single-cell sequencing of bronchoalveolar macrophages recently revealed an up-regulation of *HLA-DRB5* MHC molecules on macrophages from progressive patients compared with remitting sarcoidosis patients (Liao et al., 2021). Hence, the use of such deep sequencing technology, together with methodology used in Greaves et al. (2021), may yield a new set of progressive disease-associated MHC molecules, TCRs, and peptide antigens in sarcoidosis. Ultimately, a combination of individual MHC haplotypes, environmental triggers such as *A. nidulans*, and other genetic susceptibilities that influence cellular processes in T cells,

dendritic cells, and macrophages (Pacheco et al., 2020) may cause sarcoid disease and determine chronicity (see figure).

The elegant and comprehensive study to identify an *A. nidulans* peptide as a new sarcoid antigen also has direct clinical implications, because it validates previous studies that promote the use of antifungal therapy as an alternative to corticosteroids (Terčelj et al., 2011). Future trials testing the efficacy of antifungal therapy in *HLA-DR3*<sup>+</sup> and *HLA-DR3*<sup>-</sup> LS and non-LS sarcoidosis patients will be informative in the use of fungicides as the default corticosteroid-saving therapy for acute sarcoidosis patients.

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