INFECTIOUS DISEASE

Methylation makes a difference

Many antigens are heavily glycosylated; however, the peptides that are presented by classical MHC class I and class II molecules are not usually post-translationally modified. Now, a study published in *Nature Medicine* shows that methylation of the *Mycobacterium tuberculosis* antigen heparin-binding haemagglutinin (HBHA) is crucial for the induction of protective T-cell immunity to this pathogen.

HBHA is a cell-surface protein antigen, and the native protein (nHBHA) is post-translationally modified by methylation of lysine-rich repeats in the carboxy-terminal domain; however, recombinant HBHA (rHBHA) produced by *Escherichia coli* is not methylated. Because T cells that are isolated from healthy humans infected with *M. tuberculosis* produce large amounts of interferon-γ (IFN-γ) in response to HBHA, whereas T cells from patients with active tuberculosis do not, Temmerman *et al.* investigated the role of HBHA methylation in the immune response to *M. tuberculosis*.

Peripheral-blood mononuclear cells (PBMCs) from healthy individuals infected with *M. tuberculosis* secreted greater levels of IFN-γ in response to nHBHA than in response to rHBHA, and the proportion of individuals with nHBHA-responsive PBMCs was much greater for the healthy infected individuals than for patients with tuberculosis. The importance of methylation was confirmed by the observation that IFN-γ production was enhanced when rHBHA was methylated *in vitro* and by the finding that splenocytes from mice infected with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) produced considerably more IFN-γ in response to nHBHA than in response to rHBHA.

Importantly, the response induced by nHBHA and rHBHA, despite differing in magnitude, was mediated by both MHC-class-II-restricted $CD4^+$ T cells and classical MHC-class-I-restricted $CD8^+$ T cells. Further analysis using a panel of sequential, overlapping peptides that spanned the HBHA sequence provided some evidence to support the hypothesis that the methylated portion of HBHA forms part of the T-cell epitopes.

IFN-γ is crucial for defence against mycobacterial infection, so the protective potential of nHBHA was assessed. In two mouse models of disease, animals immunized with nHBHA and adjuvant and then challenged with a virulent strain of *M. tuberculosis* were protected from disease to the same extent as animals vaccinated with BCG, whereas mice immunized with rHBHA were not protected. The distinct abilities of nHBHA and rHBHA to induce protection correlated with splenocytes from nHBHA-immunized mice producing considerably more IFN-γ in response to bone-marrow-derived macrophages pulsed with *M. tuberculosis* and more-efficiently lysing BCG-loaded macrophages.

This report indicates that HBHA methylation modifies the cellular immune response that it elicits. Because HBHA methylation is essential for the induction of a protective immune response against *M. tuberculosis* that is comparable with the response generated by vaccination with BCG, the authors suggest that this antigen could be used to generate new vaccines against tuberculosis.

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References and links

ORIGINAL RESEARCH PAPER Temmerman, S. *et al.* Methylation-dependent T cell immunity to *Mycobacterium tuberculosis* heparin-binding hemagglutinin. *Nature Med.* 8 August 2004 (doi:10/1038/nm1090).