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UNDERSTANDING IMMUNITY TO TUBERCULOSIS : GUIDELINES FOR RATIONAL VACCINE DEVELOPMENT

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Mycobacterium tuberculosis has chosen macrophages as its preferred habitat. The tubercle bacillus is capable of arresting phagosomal maturation at an early stage, and of resisting anti-bacterial effector mechanisms, enabling *M. tuberculosis* to persist in resting macrophages[1]. Upon activation by T lymphocytes, macrophages express increased anti-bacterial activities. The activated macrophages are major effector cells of the host defense against tuberculosis, and achieve mycobacterial containment in distinct granulomatous lesions. Although containment fails to fully eradicate the pathogen in most cases, it is generally sufficient to prevent the outbreak of clinical disease. Accordingly, T lymphocytes are central mediators of the specific immune response against tuberculosis.

The T cell compartment comprises several populations that together contribute to the control of *M. tuberculosis*[1]. Of major importance are major histocompatibility complex (MHC) class Ⅱ-restricted CD4 T cells. In addition, MHC class Ⅰ-restricted CD8 T cells, $\gamma\delta$ T cells and CD1-restricted T cells, participate in protection. *M. tuberculosis* modifies the early phagosome to its advantage. It prevents phagosomal maturation at an early stage and fusion with lysosomes. Mycobacterial proteins are shuttled by MHC class I molecules from the phagosome to the cell surface, stimulating CD4 T cells. These T cells produce cytokines of Th1 type, in particular interferon-γ (IFN-γ). IFN-γ is a major activator of antimicrobial capacities of macrophages, and therefore critical for protection against tuberculosis. Other cytokines are also required for protection. *In vivo* protection focuses on granulomas, where *M. tuberculosis* is contained. Recent experiments have revealed a role of tumor necrosis factor-α (TNF-α) and lymphotoxin-α3 in granuloma formation and mycobacterial containment within a productive granuloma[2)3)]. Although it is not fully understood how mycobacteria enter the MHC class I pathway, CD8 T cells clearly do participate in protection. This notion was originally based on experiments with β2-microglobulin (β2m) knockout (KO) mice[4]. These mutant mice lack MHC class I expression and hence the functional corollary in the form

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of CD8 T cells. Recent findings reveal that β2m has several functions and therefore deficiency causes a range of effects. KO mice lacking MHC class I expression (and therefore only CD8 T cells) are less susceptible than β2m deficient mutants[5]. This demonstrates not only that CD8 T cells indeed do participate in protection against tuberculosis, but also that susceptibility due to β2m deficiency is attributable to a number of reasons.

CD8 T cells can produce IFN-γ, but more importantly, express cytolytic activities. They can lyse infected macrophages and directly attack mycobacteria[6]. It is therefore likely that cytolytic T cell functions contribute to protection against tuberculosis. Cognates of group 1 CD1 protein family present glycolipids that are abundant in the mycobacterial cell wall[7]. Group 1 CD1 molecules are present in guinea pigs and humans but not in mice, hampering accurate analysis of their role in protection. γδ T cells expressing the Vγ2 δ2 chain combination react with phospholigands abundant in mycobacteria[8]. They probably recognize phospholigands in the absence of any host presentation molecules. Both CD1 restricted and γδ T cells produce IFN-γ and express cytolytic activity and can contribute to protection against tuberculosis in this manner.

Because of the central role of the T cell compartment in protective immunity against tuberculosis, it represents a major target for activation by a vaccine[9].

Natural infection with *M. tuberculosis* is well controlled in the 2 billion individuals infected with this pathogen[9]. Annually, 8 million of the infected individuals develop tuberculosis, often after long incubation periods, during which dormant mycobacteria are controlled by the immune system and do not cause any clinical disease. Disease develops due to exogenous insult, intrinsic genetic susceptibility or both. Since the vast majority of the population do control *M. tuberculosis* in a satisfactory manner, rational vaccine design can learn from the immune response in those individuals. Importantly, the immune response in the susceptible individuals needs to be also studied to gain understanding about which mechanisms are lacking in these individuals, and are therefore critical in protection against tuberculosis. The existing vaccine, Bacille Calmette-Guérin, is of insufficient efficacy due to its failure to provide adequate protection against pulmonary tuberculosis in adults. It is important to define the mechanisms that BCG and natural infection fail to activate, in order to design novel vaccines better than BCG.

The elucidation of the genome of *M. tuberculosis* provided the blue print for the identification of candidate protective antigens and virulence factors[10]. Identification of protective antigens is particularly important for the subunit vaccine approach, while identification of virulence factors is particularly important for the whole bacterial vaccine approach.

The subunit vaccine approach includes: (1) formulations comprising protein antigens with adjuvants capable of improving immunogenicity of vaccine antigens. While such vaccines are capable of stimulating CD4 T cell responses, they often fail to satisfactorily activate CD8 T cells and unconventional T cells. (2) Naked DNA vaccines have shown promise in mouse models, as they activate both CD4 and CD8 T cells. (3) Recombinant vaccines are viable carriers expressing mycobacterial antigens and are able to stimulate both CD4 and CD8 T cells. All types of subunit vaccines stimulate a restricted number of T cell clones as they are comprised of only one or a few antigens. Unconventional T cells with specificity for non-proteinaceous antigens would be stimulated insufficiently, if at all. Promising subunit vaccine formulations, comprising protein-adjuvant utilize Antigen 85 (Ag 85), Mtb 8.4 or a fusion protein consisting of Ag85 and ESAT-6[11)~13)]. Promising naked DNA vaccine candidates include Hsp60, Ag85 and Mtb 8.4[12) 14) 15)]. Notably, successful therapeutic vaccination with naked DNA composed of Hsp60 has been reported[16)]. Recombinant Vaccinia and *Salmonella* carriers expressing Ag85 also show promise[17) 18)].

The live attenuated bacterial vaccine approach includes: (1) Deletion mutants of *M. tuberculosis*.

These should lack not only "classical" virulence factors, but also those that may impair the ensuing immune response. Promising *M. tuberculosis* mutants have been generated. They lack α-crystallin, erp, isocitrate lyase, mycolic-acid cyclopropane synthase or phthiocerol dimycocerosate[19)~23)]. (2) Auxotrophic mutants of *M. tuberculosis* or BCG. Auxotrophic mutants of BCG benefit from improved safety. Auxotrophic BCG mutants for methionine, leucine and isolysine, as well as *M. tuberculosis* auxotrophic mutants for methionine, proline and tryptophane have been described[24)25)]. (3) Recombinant BCG strains with improved immunogenicity. The weak immunostimulatory capacity of BCG could be improved by the introduction of a cytolysin, which facilitates MHC class I antigen processing[26)]. Alternatively, recombinant BCG strains expressing cytokines have been constructed[27)]. (4) Recombinant BCG over-expressing important antigens should also be considered. A recombinant BCG strain over-expressing Ag 85 has achieved better protection than wild-type BCG[28)].

By combining different approaches, further improvements could be achieved, for example, prime-boost regimens with viable bacterial vaccines and a subsequent boost with a subunit vaccine, or vice versa. As BCG vaccination will be continued also after the introduction of a novel vaccine candidate, prime boost regimes using both BCG and the novel candidate should be carefully considered.

Learning from the immune response induced by BCG vaccination and by natural infection with *M. tuberculosis*, and comparing responses between susceptible and resistant individuals will further increase our knowledge about protective mechanisms against tuberculosis. Using global transcriptome analysis will facilitate the characterization of the protective "signature" of surrogates of protection. These studies will be performed both in the human system and in various animal models, mostly mice, but also guinea pigs and non-human primates. In summary, although we are far from considering a selected vaccine candidate, recent achievements in genomics, proteomics and immunology have paved the way for the rational development of a vaccine more satisfactory than BCG[10)29)].

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〈要旨〉結核免疫の理解とワクチン開発への指針

結核菌は初期の段階でエンドソームの成熟を停止させ抗菌作用に抵抗することでマクロファージ内での増殖を可能にする。T細胞は結核特異免疫の中心的なメディエーターであり，マクロファージを活性化することによって結核感染防御を誘導する。結核菌の蛋白抗原はMHCクラスII分子によってCD4T細胞に提示され，これを活性化しIFN-γ産生を誘導する。CD8T細胞もIFN-γを産生し，細胞傷害活性を示すことで結核感染防御に重要である。脂質抗原に対応するγδT細胞などのunconventional T細胞はIFN-γ産生および細胞傷害活性を介して作用する。

結核感染は人類の約1/3にみられるが，多くは感染がコントロールされ発病しない。既に存在するBCGワクチンは成人においては適当な防御活性を賦与できないため十分ではない。新たなワクチンを開発するためには，何故BCGや自然感染が十分な感染防御活性を誘導できないか明らかにする必要がある。また，結核菌のゲノム解読によって感染防御抗原や病原因子の解明に道を開いている。防御抗原の同定はサブユニットワクチンの開発に重要であり，病原因子の同定は全菌ワクチンのために重要である。

サブユニットワクチン開発には，(1) ワクチン抗原の免疫原性を高めるためのフォーミュレーション，(2) DNAワクチン，(3) リコンビナントワクチンに関する研究が含まれる。有望なサブユニットワクチンには蛋白性アジュバントを用いたAg85やMtb8.4，あるいはAg85やESAT-6を含んだ融合蛋白，DNAワクチン候補にはHsp60，Ag85，Mtb8.4があげられる。弱毒ワクチンとしては，(1) 病原遺伝子を欠失させた結核菌ワクチン，(2) 結核菌またはBCGの栄養要求性変異株，(3) cytolysinなどを組み入れて免疫原性を高めたリコンビナントBCG株，そして (4) Ag85のような重要な抗原を過剰発現させたリコンビナントBCG株の開発が考えられている。また，異なったアプローチを組み合わせることで増強効果が期待できる。例えば，初回免疫を生菌，追加免疫をサブユニットワクチンで，あるいは初回免疫をBCGと新たなワクチンを組み合わせることでワクチン効果を高めることができるかもしれない。

BCGワクチンや自然感染後の免疫応答に関する研究や低感受性および高感受性宿主間の比較検討から結核感染防御機構に関するわれわれの知見が蓄積されつつある。有効なワクチン開発までまだまだ道のりは遠いが，最近のゲノミクス，プロテオミクス，免疫学研究によってBCGに勝る新たな結核ワクチンの登場する日が来ることを期待したい。