第 74 回総会招待講演

The 74th Annual Meeting Invitation Lecture

TUBERCULOSIS : RECENT PROGRESS IN BASIC IMMUNITY AND VACCINE DEVELOPMENT

*Ian M. ORME

*Mycobacteria Research Laboratories, Department of Microbiology, Colorado State University

Tuberculosis continues to be the most prevalent cause of death from an infectious agent globally, and its interaction with HIV is having devastating effects, particularly in Sub-Saharan Africa. Over the past decade, my laboratory has developed small animal models of pulmonary infection, which have revealed new information regarding the nature of acquired immunity, and subsequent immunopathology, in the lungs. We propose that cell mediated immunity comprises two separate elements ; protective immunity, driven by IL-12 and IFN ; and DTH, mediated by TNF and driven by chemokines. The generation of a CD 4 response is critical to both processes, but other cells are also involved in the overall control of the infection. These include gamma delta T cells, which we believe control the inflammatory influx of cells ; CD 4 + NK cells, which may play a role in focussing lymphocytes into lung granulomas ; and CD 8 T cells, which play a currently undefined role after initial expression of immunity and establishment of chronic disease in the lungs has ensued. Complex interactions between these populations of cells appear to control the influx of mediator cells into the lungs and then focus them at sites of infection. Prior to adequate expression of protective immunity the correct expression of chemokine and adhesion molecules is critical. A better understanding of these processes will hopefully in turn lead to better vaccine design, a topic which is also addressed in this paper.

1. Early events in the expression of immunity to tuberculosis in the lungs

The sequence of events that occur after a water droplet containing a tuberculosis bacterium enters the alveolar region of the lungs is very poorly understood. We surmise that when the droplet interacts with the surfactant layer the bacterium is released and phagocytosed by an alveolar macrophage. This phagocytic event is probably augmented by a variety of potential interactions between macrophage receptors, intermediary molecules such as complement components, mannose-binding protein, collectins, and so forth, and mannose residues on the mycobacterial surface[1)~3)].

The bacterium is then most likely killed, either directly by the macrophage, or indirectly by migration of the cell to the gullet and then the stomach. If it survives however there is now the potential for infection, which may be further augmented by the propensity of the alveo-

* Fort Collins CO 80523, USA.
(Received 25 Nov. 1999)

lar macrophage to adhere tightly to the alveolar surface and extend its cell membrane. If the bacterium replicates here and is able to burst the cell membrane of the macrophage this may allow erosion into the interstitium between the alveolus and the adjoining blood capillary. Normally this is essentially just a thin basement membrane, but if irritated it can swell with tissue fluid. This allows other local macrophages to enter the site, but it also provides bacteria with a possible escape route into the bloodstream[4].

As this interstitial pneumonitis develops more local macrophages arrive, and these may include dendritic cells. These cells can engulf mycobacteria, and it is possible that these cells migrate back to the regional lymphoid tissues in the bronchial tree where they sensitize T cells. This may also be triggered by bacteria escaping into the blood and being phagocytosed by macrophages in the spleen and liver. Either way, in the mouse model at least, the emergence of protective T cell responses takes three or four weeks at a minimum.

The early expression of host resistance in the lungs is clearly a complex affair involving multiple mechanisms, but the central common denominator appears to be the production of gamma interferon [IFN], a cytokine that is essential to eventual protection[5)6)]. The simple act of ingestion of mycobacteria by macrophages induces production of IL-12 by this cell, and there is emerging evidence that specific lipoproteins found on the cell wall of the bacillus can augment this event[7)]. Production of IL-12 then stimulates IFN by lymphocytes, but exactly which subsets are arriving this early in the lungs is still not understood. Candidates include CD3 + CD4 − CD8 − ["double negative"] cells that have been cloned from humans and which recognize mycobacterial lipids in the context of the CD1 molecule, and the related CD4+ NK + T cell in the mouse that possibly recognizes non-classical Class Ib-like molecules[8)9)]. Production of IFN by these mechanisms will activate newly arriving monocytes in the lesion, thus comprising an early defense mechanism against the disease. An additional component may be provided by $\gamma\delta$ T cells, which do not appear to be directly protective but which seem to control the efficient construction of the developing granuloma by producing mononuclear cell-directed chemokines[10)].

2. The nature of the acquired immune response

The full expression of protective immunity resulting in control and containment of the infection depends on the generation of a TH1-type CD4 T cell response in which specifically sensitized T cells recognize protein antigens that are predominantly represented within the culture filtrate protein pool. Following stimulation by the IL-12 heterodimer, these T cells release IFN.

The importance of this pathway has been firmly demonstrated by the use of mice in which genes encoding these proteins have been disrupted by targetted homologous recombination. Thus, infection of such mice in which the IL-12, IFN, or CD4 molecules have been disrupted allows the bacterial load in the lungs to grow in an unrestrained manner[4)]. In the latter case, the rate of infection is not as acute, reflecting the presence of the innate mechanisms still intact, as described above.

3. A possible dissociation between protective immunity and DTH

Cell-mediated immunity to tuberculosis involves the control and containment of the infection, as well as the ordered formation of a granuloma. It has long been thought that the primary purpose of the latter is not to kill the bacterium, since only a few percentage of macrophages within this structure actually harbor bacteria, but instead serves to "wall-off" the remaining viable organisms to prevent them from disseminating away from the site of infection.

This classical viewpoint is now confirmed by studies in which mice lacking the ICAM-1 molecule were infected with *M. tuberculosis*. In these experiments it was shown that protective immunity was expressed in a normal manner even if macrophage influx from local arterioles was

prevented by gene disruption of ICAM. In such mice no granuloma formation was observed, and these animals were unable to mount a DTH response[11].

In view of these findings, we hypothesize that protection and DTH are different entities of cell-mediated immunity, with protective immunity involving the IL-12/IFN TH1 axis but not involving the prolonged macrophage influx that eventually gives rise to the granuloma. This latter DTH-like component does not directly require IFN [it can be seen in IFN-KO mice] and seems to involve a predominantly β-chemokine type of response involved in recruiting monocytes into the site[12]. TNF is probably the primary controlling influence, since this cytokine stimulates local tissue cells to produce such chemokines. Accordingly, we have proposed that protection is primarily a cytokine controlled event, whereas DTH is predominantly controlled by chemokines[4].

4. Immunological status during chronic disease

Approximately two months into the course of infection after aerosol exposure of mice, the bacterial load in the lungs enters into an apparently chronic state in which only minor fluctuations in bacterial numbers can be detected. At this time it is not know whether there is a balance between bacterial activity and expression of immunity, or alternatively that the surviving bacteria are in a state of dormancy or latency that persists until external parameters permit reactivation disease.

During this phase the mouse possesses CD4 T cells that express a memory phenotype [$CD44_{hi}$ $CD45RB_{lo}$ $CD62L_{lo}$] and continue to recognize filtrate protein antigens ; we have proposed these cells represent a form of immunosurveillance mechanism[13]. In addition, CD8 T cells seem to play a role at this time, in that the chronic state soon turns into progressive disease in CD8-KO mice.

The immunopathology of the lung changes gradually over the chronic disease stage, and this differs between inbred mouse strains. Some mice, such as the C57BL/6 strain only undergo pathologic changes relatively slowly and hence can enter into old age before lung lesions break down. On the other hand certain strains such as the DBA/2 and CBA undergo much more severe pathology and show evidence of reactivation disease after only 200-250 days post-aerosol. The basis of this susceptibility remains unclear, but preliminary experiments indicate that very early events in the lungs of the different mouse strains may predispose them to reactivation events much later.

5. Current progress in TB vaccine development

Over the past five years there has been increasing efforts by many laboratories to generate new TB vaccines[14)~16)]. Some areas have been successful, and others less so, but at the time of writing current research is falling into five main areas :

(1) Avirulent mycobacteria as vaccines

There has been considerable interest in using certain saprophytic mycobacteria as vaccines, as biological response modifiers, or as vectors. At the time of writing *M. microti* is undergoing animal model testing as a potential vaccine. *M. vaccae* has been promoted as a modifier of immunity to tuberculosis [promoting a reversion to a TH1 response in patients] but a controlled clinical trial in Africa found no evidence of any activity. A further approach, that of using *M. vaccae* as a vector for over-expression of the 19 kDa antigen of *M. tuberculosis* in fact resulted in worsening of the disease in mice[17]. This information is in fact useful, because it suggests certain proteins may have a negative effect and should be excluded from a vaccine.

(2) Recombinant vaccines

To date various BCG recombinant vaccines have been generated that express mammalian cytokines. These give good protection in general, but none have been shown to confer better protection that the parent BCG strain alone. A more recent highly novel approach has been to generate a recombinant BCG expressing the listeriolysin gene of *Listeria*, the idea being that this will allow BCG to escape the phagosome and thus potentially sensitize Class I

MHC-restricted mechanisms[18]. Data to date however does not indicate any movement out of the phagosome, while animal vaccine testing is still ongoing.

(3) Sub-unit vaccines

The effort here has tended to concentrate on the culture filtrate protein pool [CFP] or proteins isolated from this pool. Several laboratories have reported protection in mice and guinea pigs with CFP given in various adjuvants, and some limited success has been seen using mixtures of purified proteins such as the hsp70 heat shock protein, and the Ag85 mycolyl transferase[19)~23)]. More recently, a novel approach that generates fusion proteins is also showing some degree of success.

In guinea pigs, CFP given in the relatively mild adjuvant MPL and supplemented with the cytokine IL-2 has shown excellent long term survival and prevention of necrotic pathology, although this preparation did not reduce the bacterial load in the lungs in the same manner seen in BCG vaccinated controls[20].

(4) Auxotrophic vaccines

In this approach auxotrophic mutants have been generated both from BCG and *M. tuberculosis*. These vaccines clearly protect, but not all are cleared well, especially in guinea pigs[24)25)]. These vaccines can be regarded as "speciality vaccines" in that they would be used in special populations in which it would be advantageous for the vaccine not to be able to grow, such as HIV-positive individuals.

(5) DNA vaccines

Currently, two DNA vaccines have been shown to have protective activity. The first encodes the Ag85A protein, and the second, rather surprisingly, encodes for hsp60 [65-kDa heat shock protein][20)26)]. Both protect mice from *M. tuberculosis* infection, and the Ag85DNA vaccine prevents long term necrosis in guinea pigs.

Acknowledgements

This work was supported by NIH programs AI-40488, AI-44072, and AI-75320. I am very honored by the invitation by the Japanese Society for Tuberculosis to present this paper.

References

1) Ferguson JS, Voelker DR, McCormack FX, et al.: Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. J Immunol. 1999; 163: 312-321.

2) Kang BK, Schlesinger LS: Characterization of mannose receptor-dependent phagocytosis mediated by *Mycobacterium tuberculosis* lipoarabinomannan. Infect Immun. 1998; 66: 2769-2777.

3) Schlesinger LS: *Mycobacterium tuberculosis* and the complement system. Trends Microbiol. 1998; 6: 47-49; discussion 49-50.

4) Orme IM, Cooper AM: Cytokine/chemokine cascades in immunity to tuberculosis. Immunol Today. 1999; 20: 307-312.

5) Cooper AM, Dalton DK, Stewart TA, et al.: Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med. 1993; 178: 2243-2247.

6) Flynn JL, Chan J, Triebold KJ, et al.: An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. J Exp Med. 1993; 178: 2249-2254.

7) Brightbill HD, Libraty DH, Krutzik SR, et al.: Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science. 1999; 285: 732-736.

8) Bendelac A: CD1: presenting unusual antigens to unusual T lymphocytes [comment]. Science. 1995; 269: 185-186.

9) Sieling PA, Chatterjee D, Porcelli SA, et al.: CD1-restricted T cell recognition of microbial lipoglycan antigens [see comments]. Science. 1995; 269: 227-230.

10) D'Souza CD, Cooper AM, Frank AA, et al.: An anti-inflammatory role for gamma delta T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. J Immunol. 1997; 158: 1217-1221.

11) Johnson CM, Cooper AM, Frank AA, et al.: Adequate expression of protective immunity in the absence of granuloma formation in

Mycobacterium tuberculosis-infected mice with a disruption in the intracellular adhesion molecule 1 gene. Infect Immun. 1998 ; 66 : 1666−1670.

12) Rhoades ER, Cooper AM, Orme IM : Chemokine response in mice infected with *Mycobacterium tuberculosis*. Infect Immun. 1995 ; 63 : 3871−3877.

13) Orme IM, McMurray DN : The immune response to tuberculosis in animal models. In: Rom WN, Garay S, eds. Tuberculosis, New York, Little, Brown and Co., 1995, 269−280.

14) Orme IM, Belisle JT : TB vaccine development : after the flood. Trends Microbiol. 1999 ; 7 : 394−395.

15) Orme IM : New vaccines against tuberculosis. The status of current research. Infect Dis Clin North Am. 1999 ; 13 : 169−185, vii−viii.

16) Orme IM : Vaccination against tuberculosis : recent progress. Adv Vet Med. 1999 ; 41 : 135−143.

17) Abou-Zeid C, Gares MP, Inwald J, et al. : Induction of a type 1 immune response to a recombinant antigen from *Mycobacterium tuberculosis* expressed in *Mycobacterium vaccae*. Infect Immun. 1997 ; 65 : 1856−1862.

18) Hess J, Miko D, Catic A, et al. : *Mycobacterium bovis* Bacille Calmette-Guerin strains secreting listeriolysin of Listeria monocytogenes. Proc Natl Acad Sci USA. 1998 ; 95 : 5299−5304.

19) Andersen P : Effective vaccination of mice against *Mycobacterium tuberculosis* infection with a soluble mixture of secreted mycobacterial proteins. Infect Immun. 1994 ; 62 : 2536−2544.

20) Baldwin SL, D'Souza C, Roberts AD, et al.: Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. Infect Immun. 1998 ; 66 : 2951−2959.

21) Horwitz MA, Lee BW, Dillon BJ, et al. : Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of *Mycobacterium tuberculosis*. Proc Natl Acad Sci USA. 1995 ; 92 : 1530−1534.

22) Pal PG, Horwitz MA : Immunization with extracellular proteins of *Mycobacterium tuberculosis* induces cell-mediated immune responses and substantial protective immunity in a guinea pig model of pulmonary tuberculosis. Infect Immun. 1992 ; 60 : 4781−4792.

23) Roberts AD, Sonnenberg MG, Ordway DJ, et al. : Characteristics of protective immunity engendered by vaccination of mice with purified culture filtrate protein antigens of *Mycobacterium tuberculosis*. Immunology. 1995 ; 85 : 502−508.

24) Guleria I, Teitelbaum R, McAdam RA, et al. : Auxotrophic vaccines for tuberculosis. Nat Med. 1996 ; 2 : 334−337.

25) McAdam RA, Weisbrod TR, Martin J, et al.: *In vivo* growth characteristics of leucine and methionine auxotrophic mutants of *Mycobacterium bovis* BCG generated by transposon mutagenesis. Infect Immun. 1995 ; 63 : 1004−1012.

26) Lowrie DB, Silva CL, Colston MJ, et al. : Protection against tuberculosis by a plasmid DNA vaccine. Vaccine. 1997 ; 15 : 834−838.