Brief Definitive Report

CROSSPROTECTION AGAINST NONTUBERCULOUS MYCOBACTERIAL INFECTIONS BY *MYCOBACTERIUM TUBERCULOSIS* MEMORY IMMUNE T LYMPHOCYTES

By IAN M. ORME and FRANK M. COLLINS

From the Trudeau Institute, Saranac Lake, New York

The nontuberculous mycobacteria (NTM) comprise a large group of micro-organisms that are rarely associated with disease in humans. This situation is rapidly changing, however, as substantial numbers of patients with Acquired Immune Deficiency Syndrome (AIDS) are now being diagnosed as infected with members of the *Mycobacterium avium* complex (1, 2). To date, however, little is known concerning the precise nature of the protective cellular response to this class of mycobacterial infections, either in humans or in experimental models in the mouse (3–5). A fundamental question that arises concerning the possible development of effective vaccines against NTM is the problem of whether species-specific antigens are important in the generation of protective immunity to each of the NTM infections, or whether this can be achieved by the recognition of common antigens expressed by most or all mycobacterial strains.

The purpose of this study, therefore, was to determine whether immune T cells, acquired in response to intravenous infection with *M. tuberculosis*, could recognize antigens (protective antigens) associated with a variety of NTM infections and thus give rise to a protective cellular response. In these experiments, memory immune T cells were used as the effector T cell population so as to overcome a number of technical problems. The first consisted of the fact that actively immune T cells, generated during the early stages of the primary immune response to tuberculosis infection, are functionally short lived (I. Orme, manuscript submitted for publication), and thus would not be expected to survive long enough to give rise to a state of immunity after passive transfer into recipients infected with NTM; NTM infection sometimes can take up to 1–3 mo to grow to an immunogeneic threshold (6). Also, to prevent the possibility of contaminating viable *M. tuberculosis* being transferred in the cell inoculum (7) and actively immunizing the recipient so that any observed effects on the NTM test infection might be the result of increased nonspecific resistance (8), donor mice were given a protracted course of isoniazid chemotherapy to eliminate viable *M. tuberculosis* and thus give rise to a state of memory immunity (9) in these animals.

Our results provide evidence that strongly support the hypothesis that relevant protective antigens expressed by NTM infections can be recognized by memory immune protective T cells that have been acquired in response to *M. tuberculosis* infection, thus suggesting that these protective antigens are common, or suffi-

ciently crossreactive to give rise to the generation of a protective cellular response.

Materials and Methods

Mice.   These experiments were performed using specific pathogen–free male C57BL/6 mice 6–8 wk old. These animals were provided by the Trudeau Animal Breeding Facility.

Bacteria.   *M. tuberculosis* (strain Erdman; Trudeau Mycobacterium Collection [TMC] 107), *M. simiae* (N29; TMC 1226), *M. avium* (3459-1-T; TMC 724), and *M. kansasii* (Brownell; TMC 1203), were grown under conventional conditions previously described (10).

Experimental Infections.   Stored frozen ampoules of bacteria were thawed, briefly sonicated, and diluted to the required inoculum size in cold sterile PBS. Mice were infected intravenously with an inoculum of 0.2 ml of the bacterial suspension via a lateral tail vein.

Mice were exposed to airborne infections using a Middlebrook Airborne Infection Apparatus (Tri-R Instruments, Inc., Rockville Centre, NY). The nebulizer compartment was filled with inocula of bacteria adjusted to concentrations calibrated to deliver an acute infectious dose ($\sim 10^4$ viable bacteria) into the lungs over a 30 min exposure period (7).

The course of experimental infections was followed against time by sacrificing 4–5 animals and plating serial dilutions of individual whole target organ homogenates on nutrient Middlebrook 7H11 agar (Difco Laboratories, Detroit, MI) and counting bacterial colony formation after 14–20 d incubation at 37°C.

Passive Cell Transfers.   Groups of donor animals were infected intravenously with 10^5 *M. tuberculosis* Erdman. One group of mice was infected with 10^7 heat-killed *M. tuberculosis* as an additional control. 25 d later, mice were exposed to 200 mg/liter isoniazid (iso-nicotinic acid hydrazide; Pfizer Co., NY) in their drinking water. After 60 d more, the numbers of viable bacteria in the target organs of these mice had fallen below levels of detectability, and the animals were sacrificed and spleen cells were harvested. These cells were suspended in RPMI 1640 tissue culture medium supplemented with 1 mM glutamine and 2% heat-inactivated FCS, and then enriched for T cells by the procedure of Mage et al. (11), which depletes B cells by adherence to plastic dishes coated with antisera against mouse Ig. In some experiments, these cells were then treated with anti-Thy-1.2 mAb plus complement, as previously described (7). One spleen equivalent of memory immune T cell–enriched spleen cells were then intravenously transferred into age- and sex-matched syngeneic recipients that had been rendered T cell–deficient by exposure to 500 rad of gamma irradiation before the transfer (7). Control mice were infused with normal T cell–enriched spleen cells. To exclude the possibility that a few viable *M. tuberculosis* organisms had escaped the effects of isoniazid therapy in the donor animals, recipient mice were started on isoniazid 7 d before the transfer, and for 7 d after transfer. After this time, a few recipient mice were further checked for the presence of viable mycobacteria by treatment with cortisone acetate (9) followed by plating of target organs 14 d later; all mice were negative for the presence of active infection.

14 d after the passive transfer of memory immune T cells, test and control mice were challenged via intravenous or aerogenic routes with the various mycobacterial infections, and the courses of these infections were followed against time in target organs as above.

Results

Efficacy of Isoniazid Chemotherapy.   In initial experiments, recipient mice that had received spleen cell inocula from isoniazid-treated donor mice were checked for the presence of possible contaminating viable *M. tuberculosis* organisms; this was performed in view of the earlier observation (9) that a few residual bacteria can often survive this type of chemotherapy. We found (Table I) that if recipient animals were not treated with isoniazid, a small but potentially immunizing

TABLE I

Efficacy of Isoniazid Chemotherapy

Chemotherapy given		$\log_{10}$ bacteria in spleens on day 14[‡]
Isoniazid	Cortisone*	
−	−	3.01 ± 0.08
−	+	3.86 ± 0.33
+	−	<1.00
+	+	<1.00

* Mice were injected subcutaneously with 20 mg/kg cortisone acetate daily for 14 d.

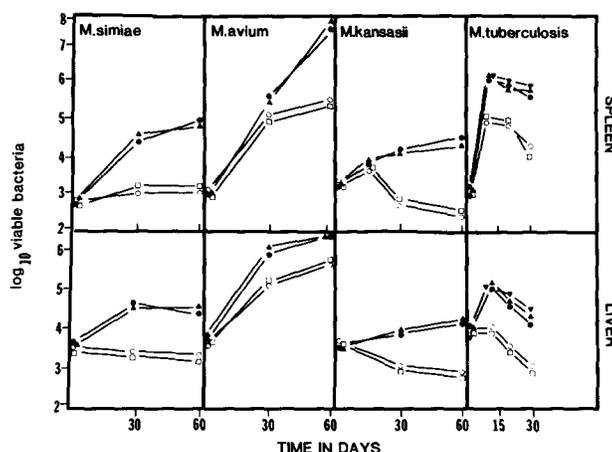[‡] Mean numbers of bacteria recovered ± SEM ($n = 4$).

FIGURE 1. The growth of four intravenous mycobacterial challenge infections in the spleens (top) and livers (bottom) of T cell–deficient mice adoptively immunized with M. tuberculosis–specific memory immune T cell–enriched spleen cells. The data shows the course of the challenge infection in recipients given memory immune cells (□), normal cells (●), or immune cells treated with anti-Thy-1.2 mAb plus complement (▲), or with complement alone (○). In addition (right), some donor mice received heat-killed M. tuberculosis (▼) instead of the living organism. Data is expressed as the mean number of bacteria in target organs ($n = 4–5$); SEM is omitted; it never exceeded 0.24.

number of viable M. tuberculosis could be recovered from the spleens of these animals 14 d later. Based upon our previous experience with this organism, the numbers of bacteria recovered after this period probably reflected ∼10–50 viable bacteria originally present in the transferred inoculum.

Treatment of similar groups of recipient mice with isoniazid destroyed contaminating bacteria to below levels of detectability (Table I). The effectiveness of this treatment was further shown by a failure to detect viable bacteria in these animals following continued injection with cortisone acetate. Thus, although it was clear that a few viable bacteria were originally present in the transferred spleen cell inocula, the possibility that these organisms survived in the isoniazid-treated recipients, thus actively immunizing these recipients, was regarded as unlikely.

Passive Transfer of Protection Against Intravenous NTM Infections.   Adoptively immunized recipients were substantially more resistant than controls to intravenous infection with M. tuberculosis (Fig. 1). This resistance was characterized by
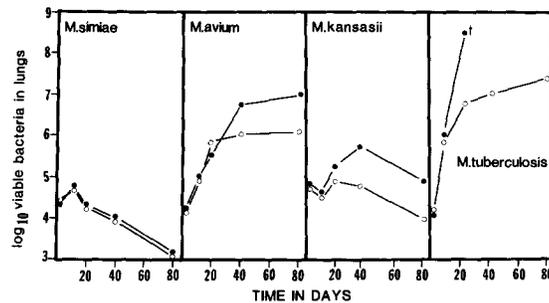
FIGURE 2. The growth of the four test mycobacterial challenge infections in the lungs of adoptively immunized (O) and control mice given normal T cells (●) following aerogenic deposition of $\sim 10^4$ mycobacteria. Mice infused with normal cells and challenged with *M. tuberculosis* (right) died by day 25. Data expressed as mean values ($n = 4$-5); SEM is omitted; it never exceeded 0.22.

a slower rate of progressive growth of the infection in the spleen, and its containment, without detectable proliferation, in the liver. Evidence for the generation of a protective cellular response was also observed against the three NTM infections, although its effect on the course of each infection differed. In the case of *M. simiae*, proliferation was completely prevented, thus resulting in an apparent bacteriostasis. In contrast, there was evidence for the slow but progressive elimination of the *M. kansasii* infection, while the third infection under test, *M. avium*, grew rapidly in the spleens and livers of the adoptively immunized animals before showing some evidence of slowing after day 30 of the experiment.

In each infection, the protective effect of the passively transferred memory immune spleen cells was mediated by T cells, as evidenced by the observation that the protective effect was ablated by prior treatment of transferred cells with anti-Thy-1.2 mAb plus complement, but not by complement alone (Fig. 1). Finally, as an additional control, recipients that had received T cell–enriched spleen cells from donor mice that had been injected with 10^7 heat-killed *M. tuberculosis* were shown to possess no enhanced resistance to the homologous viable challenge infection, thus indicating that dead mycobacteria that might be present in the transferred cell inoculum had no immunizing capacity.

Passive Transfer of Protection Against Airborne NTM Infections. In a parallel series of experiments, the capacity of adoptively immunized recipients to resist airborne infection with each NTM was determined. We found (Fig. 2) that, in three experimental infections (*M. tuberculosis*, *M. kansasii*, and *M. avium*), recipients that had received memory immune T cells were able to express significantly enhanced resistance to these challenge infections, although it was clear that in each case this resistance was expressed somewhat more slowly in the lungs than to the intravenous challenge. In the case of *M. simiae*, however, this organism was slowly cleared from the lungs of control animals, and there was no evidence of any acceleration of this process in the adoptively immunized mice.

Discussion

The results of this study show that the adoptive immunization of T cell–deficient recipients by the passive transfer of *M. tuberculosis*–specific memory

immune T–enriched spleen cells can confer upon that recipient the capacity to express enhanced resistance to both a homologous challenge and to challenge with three representative NTM infections, M. simiae, M. avium, and M. kansasii. Prior treatment with anti-Thy-1.2 mAb plus complement ablated this protective effect, thus formally showing that it was mediated by T lymphocytes.

The results of the study thus support the hypothesis that the antigenic determinants expressed by these three NTM infections which will give rise to a protective cellular response to the homologous organism, are identical or closely crossreactive to epitopes that are recognized by M. tuberculosis–specific memory immune T cells. These observations, therefore, may help to explain the mechanism by which M. bovis BCG vaccination can be effective against NTM infections (12), which presumably reflects another example of cross-protective specific immunity.

The nature of the protective antigens that give rise to cellular immunity to M. tuberculosis and the NTM infections, however, remains totally unknown. The effective generation of protective immunity to these organisms requires the presence of living bacteria, and, while heat-killed mycobacteria may, in some situations, increase the nonspecific resistance of the host, no formal evidence for the generation of protective T cells in this latter case is currently available. In view of this, it is tempting to speculate that the protective antigens that appear to be common to the immunogenic mycobacteria may be metabolites that are produced by the living proliferating organism, rather than being structural antigens.

Summary

Adoptive immunization of T cell–deficient recipient mice with M. tuberculosis–specific memory immune T lymphocytes conferred upon these animals the ability to express significantly enhanced resistance both to the homologous infection, and to three strains of nontuberculous mycobacteria. These results support the hypothesis, therefore, that antigenic determinants possessed by the four mycobacterial strains that are relevant to the generation of protective cellular immunity are identical or closely crossreactive.

References

1. Zakowski, P., S. Fligiel, G. W. Berlin, and L. Johnson. 1982. Disseminated Mycobacterium avium-intracellulare infection in homosexual men dying of acquired immunodeficiency. JAMA (J. Am. Med. Assoc.). 248:2980.

2. Strom, R. L., and R. P. Grunninger. 1983. AIDS with Mycobacterium avium-intracellulare lesions resembling those of Whipple's disease. N. Engl. J. Med. 309:1323.

3. Wolinksy, E. 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. 119:107.

4. Orme, I. M., and F. M. Collins. 1984. Immune response to atypical mycobacteria: Immunocompetence of heavily infected mice measured in vivo fails to substantiate immunosuppression data obtained in vitro. Infect.Immun. 43:32.

5. Collins, F. M. 1983. Kinetics of the delayed-type hypersensitivity response in tuberculous guinea pigs and mice tested with several mycobacterial antigen preparations. Am. Rev. Respir. Dis. 127:599.

6. Orme, I. M., and F. M. Collins. 1984. Efficacy of *Mycobacterium bovis* BCG vaccination in mice undergoing prior pulmonary infection with atypical mycobacteria. *Infect. Immun.* 44:28.

7. Orme, I. M., and F. M. Collins. 1983. Protection against *Mycobacterium tuberculosis* infection by adoptive immunotherapy. *J. Exp. Med.* 158:74.

8. North, R. J., G. B. Mackaness, and R. W. Elliot. 1972. The histogenesis of immunologically committed lymphocytes. *Cell. Immunol.* 3:680.

9. Lefford, M. J., and D. D. McGregor. 1974. Immunological memory in tuberculosis. I. Influence of persisting viable organisms. *Cell. Immunol.* 14:417.

10. Collins, F. M. 1971. Immunogenicity of various mycobacteria and the corresponding levels of cross-protection developed between species. *Infect. Immun.* 4:688.

11. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulins: preparative scale separation in polystyrene culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* 15:47.

12. Orme, I. M., and F. M. Collins. 1985. Prophylactic effect in mice of BCG vaccination against nontuberculous mycobacterial infections. *Tubercle.* 66:117.