Effects of microenvironment on local regulation of granulomas caused by *Mycobaterium leprae*

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INTRODUCTION

Mycobacterium leprae is an obligate intracellular pathogen and the causative agent of Hansen's Disease (HD), a disease characterized by a broad spec- trum of clinical, histopathological and immunological presentations that afford the opportunity to study immune regulation and the interplay of T cells and macrophages (MO) in a specific, nonfatal, human, chronic immunodeficiency disease. At one pole of the HD clinical disease spectrum is tuberculoid disease (TT), characterized by low levels of antibody, very few bacilli in the skin lesions and a fully competent cell mediated immunity (CMI) to *M. leprae* antigens. In sharp contrast, at the other pole of clinical HD is lepromatous disease (LL), characterized by an *M. leprae* -specific anergy in CMI, a potent antibody response and enormous numbers of *M. leprae* in the lesions.

At both poles M. *leprae* infection is local- ized to the cooler areas of the body, i.e. the skin and mucous membranes of the upper respiratory tract. HD granulomas across the spectrum are composed of numerous cell types, including an array of lymphocytes of the helper and sup- pressor phenotype, MΦ at various stages of maturity, and other cells. The present report summarizes the interest from our laboratory in the localized mechanisms and lesional microenvironmental factors that regulate the interaction between T cells and MΦ) in CMI to *M. leprae* at both poles of the HD spectrum. Our interest in control at the level of the granuloma microenvironment stems from our studies of macrophage success and failure in coping with viable HD bacilli[8] and our initial failure to demonstrate by adoptive transfer enhanced resistance to *M. leprae* in infected nu/nu mice infused with viable sensitized T cells.

MΦ Success in Coping with M. leprae

Using radiorespirometry as an objective measure of M. leprae viability[4], we demonstrated that, although viable bacilli appear to thrive in normal mouse peritoneal MΦ)[10], IFNy-activat- ed MΦ were able to markedly kill or inhibit M. leprae in vitro by microbicidal mechanisms dependent on the arginine-dependent production of NO[1]. These findings confirmed the demonstration that in normal MΦ phagosome- lysosome fusion was blocked by MΦ. leprae. In activated MΦ, phagosomes harboring M. leprae fused with secondary lysosomes[11].

Down regulation of MO Effector Function by *M. leprae*

To explore the ability of MΦ from lepromatous granulomas to cope with the HD bacillus we isolated and cultured MΦ from the enlarged foot pads of *M. leprae* -infected athymic (nu/nu mice). Foot pads of mice infected for nine to twelve months routinely yielded five to ten million macrophages engorged with *M. leprae.* These granuloma MΦ were evaluated in parallel with peritoneal MCI) from the same animals for a number of phenotypic markers and IFNy-inducible immune functions". Interestingly, except for containing enormous numbers of *M. leprae,* the granuloma MΦ) were phenotypically indistinguishable from normal peritoneal MO: they were adherent to plastic, phagocytic, supported the intracellular growth of *Toxoplasma gondii,* were nonspecific esterase positive, and possessed Fc and C3Bi receptors. However, unlike the peritoneal MΦ, the granuloma Mff) released prostaglandin E2 (PGE2) into supernatant medium. In addition, granuloma MΦ) differed markedly from the autologous peritoneal cells in their responsiveness to IFNγ (Table 1).

The *M. leprae*-engorged granuloma MΦ) were refractory to IFNγ-mediated activation for

Table 1. IFNγ-induced Effector Function in MΦ) Infected with M. *leprae* or Treated with LAM

IFNγ-induced MΦ) Effector Function	*M. leprae* infected nu/nu mice		Per. MΦ) infected *in vitro* with *M. leprae*	Per. MΦ Rx *in vitro* with LAM
	Per. MΦ	FP MΦ		
Cidal for toxoplasma	Yes	No	No	No
Cidal for *M. leprae*	Yes	No	No	No
Tumoricidal	Yes	No	No	No
Superoxide production	Yes	No	No	No
MHC Class II induced	Yes	No	No	No

both microbicidal and tumoricidal activity. In addition, there was no IFNγ-induced augmentation of Class ll MHC expression or PMA-induced superoxide production in the *M. lepra* e-engorged granuloma MΦ. These findings show that *M. leprae* can be a potent modulator of MΦ effector functions, and also demonstrate that this effect is confined to the microenvironment of the granuloma.

In vitro studies shed more light on these *ex vivo* findings[13]. Normal peritoneal MΦ infect- ed with large numbers (E:T=50:1) of viable bacilli and cultured > 48 hr also produced PGE2 and became refractory to IFNγ (Table 1) by an indomethacin reversible mechanism. Equally large numbers of killed bacilli failed to induce this effect. Additional studies have focused on an abundant mycobacterial cell wall compo- nent, lipoarabinomannan (LAM) as a potent constituent underlying the ability of *M. leprae* to down regulate MΦ) effector function[7].

Traffic of M. leprae into Lepromatous Lesions

To explore the turnover of M. *leprae* infect- ed MΦ in the nu/nu mouse foot pad lesions we pulse labeled bone marrow promonocytes and followed their traffic into the foot pads by autora- diography[9]. Remarkably these studies showed that 25-30% of the *M. leprae* gorged MΦ were less than five days old, suggesting that there is a continuous, very dynamic turnover and replacement of MΦ in such lesions. There is ample evidence that cytotoxic CD8 and CD4 T cells can lyse target MΦ infected with mycobacteria, although there is a paucity of these specific T cells in lepromatous HD (and nu/nu mice). However, we have shown that NK cells are able to lyse *M. leprae* infected MΦ target cells and that the interaction of activated effector MΦ and M. *leprae* infected target MED results in the killing or inhibition of the bacilli'.

Adoptive Transfer Experiments

To further explore the localized CMI to HD, adoptive transfer (AT) experiments were performed. Briefly, in our model nu/nu mice infected with *M. leprae* 6 months previously were infused i.v. with 2 x 10^7 sensitized lymph node T cells from immunocompetent donors immunized with *M. leprae.* As an objective indicator of successful AT, after 2 weeks we harvested the *M. leprae* from the foot pads of recipient nu/nu mice and determined their viability by radiorespirometry.

An initial series of AT experiments in *M. leprae* infected nu/nu mice yielded disappoint- ing results. As summarized in Table 2, AT of sensitized T cells failed to adversely effect the viability of M. *leprae* from the foot pads of recipient nu/nu mice. Because of the potent down regulating effects of PGE2 in CM! in general and its role in our *in vitro* and *ex vivo* studies of down regulation of the effector function in *M. leprae* infected MΦ, subsequent AT experiments were designed to focus on the role of PGE2 in modulating local CMI to *M. leprae.*

PGE2 is synthesized from arachidonic acid (AA), one of the major phospholipids found

in cell membranes, via the cyclooxygenase (COX) pathway. AA is generated from linoleic acid, a plant derived, essential fatty acid (EFA) provided by diet. Feeding mice a defined diet containing all necessary nutrients except linoleic acid greatly reduces the levels of AA in the tis- suess, thus depriving COX of its substrate and blocking COX-mediated biosynthesis of PGE2. To test the importance of localized PGE2 production in AT infected nu/nu mice were fed EFA sufficient (S) or deficient (D) diets for 3 months prior to infusion with normal or sensitized T cells. The results (Table 2) clearly showed that the AT of *M. leprae* sensitized T cells resulted in killing or inhibition of HD bacilli in the foot pads of recipient mice on EFAD diets in comparison to mice on EFAS diets, presumably by activated MΦ.

Table 2. Viability of *M. leprae* isolated from nu/nu foot pads two weeks after adoptive transfer of T cells.

NuMu Diet	Adoptively Transfered T Cells	Viabile *M. leprae* from foot pad[a]
Normal Chow	None	3/3 mice
	Sensitized T cells	3/3 (exp A) 7/7 (exp B)
EFAS	Normal T cells	8/8 mice
	Sensitized T cell	5/8 mice
EFAD	None	3/3 mice
	Normal T cells	7/7 mice
	Sensitized T cells	2/11 mice

[a]Viability determined by radiorespirometry

Summary

Overall, our results suggest that induction of enhanced local production of PGE2 by *M. leprae* infected MO, while having little influence on the cells in the rest of the body, could modulate considerably the function of the surrounding cells, both T cells and MΦ We have singled out for study PGE2 although other COX products could be involved as well. Obviously, the immunoregulatory mechanisms operative in CMI to HD are more complex than presented here; numerous monokines, cytokines, chemokines and other cell products are likely involved.

Collectively, our findings that lepromatous MO are refractory to IFNγ activation accomodate the results of experiments that showed the upgrading of LL lesions in HD patients treated intralesionally with human IFNγ6. We have demonstrated that MΦ can be activated before infection with *M. leprae* and although we have shown that MΦ production of PGE2 may under- lie the refractory state to IFNγ activation in heavily infected MO there is a delay before exogenous PGE2 down regulates MΦ function. Since the LL granuloma is a very dynamic lesion with active turnover of infected MO, we suggest that the human IFNγ immunotherapy findings are a consequence of the activation of new MΦ before or shortly after their traffic into the lesion.

REFERENCES

1. ADAMS, L. B.; FRANZBLAU, S. G. et al. L-arginine-dependent macrophage effector functions inhibit metabolic activity of *Mycobacterium lep- rae.* **J. Immunol**. 147:1642-1646, 1991.

2. ADAMS, L.; FUKUTOMI, Y. et al. In vitro study of macrophage turnover in experimental leprosy. Presented at the Twenty-Sixth Joint Leprosy Conference sponsored by the U.S. and Japanese Panels of the U.S.-Japan Cooperative Medical Science Program, Seattle, Washington, 6-9 August 1991.

3. BONTA, I. L.; PARNHAM, M. J. et al. Reduced exudation and increased tissue proliferation during chronic inflammation in rats deprived of endogenous prostaglandin precursors. **Prostaglandins** 14:295-306, 1977.

4. FRANZBLAU, S. G. Drug susceptibility testing of *Mycobacterium leprae* in the BACTEC 460 sys- tem. **Antimicrob. Agents Chemother**. 33:2115-2117, 1989.

5. GU, L.; KRAHENBUHL, J.L. Induction of NK/LAK-like cells activity in the peritoneal cavity of mice by *Mycobacterium leprae.* **Chinese J. Microbiol. & Immunol**. 14:22-25, 1994.

6. KAPLAN G.; MATHUR N. et al. Effect of multiple interferon gamma injections on the dispos- al of *Mycobacterium leprae.* **Proceedings of the National Academy of Science USA** 86: 8073-8077 1989.

7. KRAHENBUHL, J.L. Chapter entitled 'Role of Mycobacterial Constiuents in Regulation of Macrophage Effector Function'. *In* Virulence Mechanisms for Bacterial Pathogens (Roth, ed.), Impressions, a Division of Edwards Brothers, Inc., Madiscon, WI, Publishers, 1995.

8. KRAHENBUHL, J.L.; ADAMS, L. B. The interaction between the macrophage and the leprosy bacillus. *In* Macrophage-Pathogen Interactions, pp. 281-302. B. Zwilling and T. Eisenstein, eds., Marcel-Dekker, 1993.

9. KRAHENBUHL, J. L.; SIBLEY, L. D. et al. y-Interferon in experimental leprosy. **Diagn. Microbiol. Infect**. Dis. 13:405-409, 1990.

10. RAMASESH, N.; ADAMS, L. B. et al. Effects of activated macrophages on *Mycobacterium leprae.* **Infect. Immun**. 59:2864-2869, 1991.

11. SIBLEY L. D.; FRANZBLAU S. G. at al. Intracellular fate of *Mycobacterium leprae* in normal and activated mouse macrophages. **Infection and Immun**. 55(3): 680-685, 1987.

12. SIBLEY, L. D.; KRAHENBUHL, J. L. Defective activation of granuloma macrophages from *Mycobacterium* leprae-infected nude mice. **J. Leukoc. Biol**. 43:60-66, 1988.

13. SIBLEY, L. D.; KRAHENBUHL, J. L. Induction of unresponsiveness to gamma interferon in macrophages infected with *Mycobacterium leprae.* **Infect. Immun**. 56:1912-1919, 1988.