Activation of complement by *Mycobacterium leprae* requires disruption of the bacilli

RAMANUJ LAHIRI, FELIPE G. SANDOVAL, JAMES L. KRAHENBUHL & EDWARD J. SHANNON

Laboratory Research Branch, National Hansen’s Disease Programs, Louisiana State University, Baton Rouge, 70803 U.S.A.

Accepted for publication 30 April 2008

Summary

Objective The immune-mediated events that precipitate erythema nodosum lepromatous leprosy (ENL) are not well understood. One component may be the complexing of antibody with antigens released from infected macrophages, the activation of complement and the subsequent local inflammation. We assess here the ability of highly-purified, disrupted *M. leprae*, to activate complement.

Results Intact and sonically-disrupted crude and alkali-purified nu/nu mouse-derived *M. leprae* suspensions were incubated with normal serum and a hemolytic titer (CH50) was determined as a measure of complement fixation. Crude *M. leprae* consumed complement, and disrupted preparations more than the intact. Purified *M. leprae* preparations did not consume complement unless disrupted.

Conclusion *M. leprae*, if disrupted, can activate complement. This supports a hypothesis that links released antigens with ENL, and may explain the increased probability of an occurrence of ENL following chemotherapy.

Introduction

Globally leprosy still remains a major public health problem owing to the deformities caused by this disease. Erythema nodosum lepromatous leprosy (ENL) is a debilitating reaction that may occur in lepromatous and borderline lepromatous leprosy patients. The sequence of immune mediated events which precipitate ENL, have not yet been clearly delineated, but likely include both humoral and cell mediated mechanisms as summarised in a recent review.1 The present study is concerned with one potential mechanism.

The leprosy patient with ENL is characterised by an abundance of circulating precipitating antibodies and huge numbers of *M. leprae* inside macrophages.2 As the likelihood of an episode of ENL increases after the initiation of treatment3 and many of
the histological features of ENL resemble an experimentally induced Arthus reaction in animals, it has been suggested that release of M. leprae antigens from killed bacilli within macrophages may be an initiating factor. The released antigen may then complex with antibodies, initiating complement fixation and production of neutrophil chemotactins like C3a and C5a. Depositions of immunoglobulin and complement in acute ENL lesions have been observed and evidence supporting in vivo activation of complement during ENL has been described by several investigators.

In this study, we determined the level of complement fixation using disrupted M. leprae, as a model for released antigens in treated individuals and compared that level of complement fixation to live, intact, bacteria. We incubated normal human sera with either intact or sonically disrupted M. leprae and then determined if complement had been consumed. Our results show that disrupted M. leprae can significantly activate complement.

Materials and Methods

Our method was to harvest M. leprae (Thai-53 isolate) from the foot pads of athymic nu/nu mice, and adjust them to 1x10⁹ AFB /ml in RPMI-1640. This preparation was identified as crude M. leprae. In parallel an aliquot of the crude M. leprae preparation was treated with 0·1N NaOH (Sigma), and identified as pure viable intact M. leprae. Aliquots of each of crude and pure intact M. leprae were disrupted by intermittent pulsing using a probe sonicator, and were identified as pure and crude sonicated M. leprae respectively. Disruption of the bacteria was confirmed by microscopic examination. For determination of CH50, a slight modification of the Diamedix EZ Complement CH50 TEST (Diamedix Corporation) was used. Briefly, 100 µL of the high CH50 titer reference sera (our source of normal sera for all of the assays) was incubated for 1 hr at 33°C with 100 µL from each of the four M. leprae preparations. Then 5 µL of the supernatant was incubated with sheep erythrocytes, sensitised with antibodies to sheep erythrocytes. After 1 hour at room temperature the incubation vessel was centrifuged and the OD of the supernatant determined at 405 nm.

Results

The data illustrated in the Figure are a representation one of five experiments (Figure 1).

Mann-Whitney rank sum test was used to determine significance between the observed differences of medians. P < 0.05 was taken as significant. Compared to the RPMI Control (E), the pure viable intact M. leprae (C) did not fix complement (P = 0.112). Both intact and sonicated preparations of crude M. leprae (A, B) consumed complement (P < 0.001), the latter more markedly than the former. Of special interest is the observation that the pure sonicated M. leprae (D) consumed more complement than the pure viable intact M. leprae (C) (P = 0.005) as well as the RPMI Control (E) (P = 0.004).

Discussion and Conclusions

Evidence supporting complement fixation as the basis of ENL reactions is essentially indirect, derived from patients’ sera and biopsied tissues. In vitro, M. leprae has been
reported previously to activate complement by both the classical and alternate pathways. However, these findings may have been compromised by an artifact in the reagent employed. When *M. leprae* is harvested, by a two-phase separation procedure, from *M. leprae*-infected armadillo tissues, it contains significant remnants of armadillo tissue (unpublished scanning electron microscope observations). Subcellular membranes (mitochondrial and microsomal) in host tissue are known to activate complement by both the classical and alternative pathways, a characteristic that we feel may underlie these reported findings and the marked consumption of complement seen in the present study when crude intact nu/nu mouse derived *M. leprae* was employed.

The most interesting outcome of the present study was the demonstration that disrupted *M. leprae* was capable of enhanced complement fixation when compared to intact bacilli. We would like to underscore the fact that our reagents (intact and disrupted bacilli) for each of five replicate experiments originated from freshly harvested different suspensions of nu/nu mouse derived *M. leprae*. Our freshly harvested, NaOH purified nu/nu derived *M. leprae* are a unique reagent and previous studies have shown that *M. leprae* suspensions, prepared by our method, remain viable and are virtually free of mouse tissue after NaOH treatment. The study also underscores the importance to researchers of characterising, to the best of their ability, the nature of the preparations of *M. leprae*, they are using for in vitro experiments.

Our studies indicate complement fixation in the presence of disrupted, pure *M. leprae*. These findings support a hypothesis linking the killing and breakdown of intact leprosy bacilli during chemotherapy with released antigens that initiate complement activation and subsequent ENL reactions. While these in vitro findings bolster this as a putative mechanism for ENL, they remain to be confirmed in the clinical setting of this reaction.

**Acknowledgements**

The authors are grateful to Baljit Randhawa and J. P. Pasqua for their excellent technical help.
References