FACTORS INVOLVED IN IMMUNITY TO *NEMATOSPIROIDES DUBIUS* INFECTIONS IN MICE

by

Varunee Desakorn
B.Sc.Med.Tech. (Thailand)
M.P.H. (Philippines)

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ABSTRACT

The present investigation examined the interaction between mouse peritoneal macrophages with various developmental forms of Nematospiroides dubius (Heligmosomoides polygyrus) a natural nematode parasite of the mouse and the ability of serum from mice immune to this infection to damage exsheathed L_3 in the absence of phagocytic cells.

Initial studies examined the adherence of peritoneal exudate cells to the surface of the parasite and the factors involved in promoting this adhesion. Peritoneal exudate cells from normal mice did not adhere to the cuticle of exsheathed L_3 in the absence of serum, but did so when the larvae had been sensitised with normal mouse serum. In this case the cells bound to the larvae via their C3 receptors and confirmed previous observations that the parasite activated complement via the alternative pathway. It was also found that serum from mice immune to this infection contained antibodies directed against the cuticle of all larval stages as measured by the adhesion of cells to larvae that had been sensitised with heat inactivated serum. The cells in this instance adhered to the larvae via their Fc receptors. The factors involved in promoting adhesion of peritoneal exudate cells from normal mice and those from mice resistant to this infection were similar. The peritoneal exudate cells adhering to the larvae were found to be predominantly macrophages.

The cell adhesion studies demonstrated that all of the developing post-infective and the adult form of the parasite were capable of activating complement by the alternative pathway with the marked exception of larvae collected 96 hours post-infection. This

indicated a possible change in the antigenic structure of the cuticle.

Experiments were carried out to determine the ability of various immunoglobulin isotypes from mouse immune to reinfection to mediate cell binding to all developmental forms and adult worms of the parasite. It was found that the ability of IgM to promote the binding of cells to the larval stages increased dramatically from the pre-infective stages including sheathed and exsheathed L_3 up to 96 hours post-infective larvae, but promoted little binding to adult worms. The IgG_1 promoted cell binding in a similar pattern to that of IgM, but binding of the cells to 96 hours post-infective larvae was considerably reduced. Both IgG_{2a} and IgG_{2b} enhanced the binding of cells to pre-infective larvae, but were not active in promoting cell binding to post-infective larvae. However at a physiological level only IgG_1 and IgM were active in promoting cell adhesion.

An in vitro assay to determine the effect of cell binding upon the infectivity of exsheathed L_3 indicated that peritoneal exudate cells from mice infected with 2 doses of L_3 were capable of damaging the larvae (as measured by a loss in infectivity) providing the larvae had been sensitised with antibody and complement or complement alone, but had no effect if the larvae had been sensitised with antibody alone even though antibody mediated cell adherence. Peritoneal exudate cells from normal mice were unable to damage the larvae even in the presence of antibody and complement.

Further in vitro studies showed that fresh untreated serum from mice infected with 4 doses of L_3, IMS(4) was able to damage exsheathed L_3 as measured by a reduction in their infectivity.

This effect was not apparent when fresh untreated serum from mice given 2 immunising doses, IMS(2) was used. The larvicidal activity was found only in long-term N. dubius infected mice since serum from mice infected with Mesocestoides corti an unrelated helminth parasite did not reduce the infectivity of the larvae. The larvicidal activity of IMS(4) appeared to be dependent on specific antibodies of the IgM class reacting with the cuticle of the larvae and binding complement.

Studies in vivo showed that fresh IMS(4) given intravenously to naive mice protected them against a subsequent intravenous challenge with exsheathed L_3, but not against a challenge with sheathed L_3. It was also found that serum from immune mice from which the greater proportion of the immunoglobulins had been removed was unable to transfer immunity passively to naive mice. The relative importance of these studies to other investigations dealing with nematode parasites is discussed.

STATEMENT

This thesis contains no material previously submitted by me for a degree in any university, and to the best of my knowledge and belief it contains no material previously published or written by another person, except where reference is made in the text.

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ABBREVIATIONS USED IN THIS THESIS

BSA	Bovine serum albumin
c.a.	approximately
EDTA	ethylenediaminetetraacetate
EGTA	ethyleneglycol-bis (β-aminoethyl) N,N'-tetraacetate
ELISA	Enzyme-linked immuno-sorbent assay
Ig	Immunoglobulin
IMS	immune mouse serum
IMS(2)	serum from 2 times *N. dubius* infected mice
IMS(4)	serum from 4 times *N. dubius* infected mice
L_3	Third-stage (infective) *N. dubius* larvae
N.D.	not done
NMS	normal mouse serum
PBS	Phosphate-buffered saline
tris	Tris(hydroxymethyl)aminomethane
VBS	Ca^{++}, Mg^{++} - supplemented veronal-buffered saline

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