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Immunity to *Trichuris muris* in the laboratory mouse

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Abstract

Of all the laboratory models of intestinal nematode infection, *Trichuris muris* in the mouse is arguably the most powerful. This is largely due to the fact that the ability to expel this parasite is strain dependent. Thus, most mouse strains readily expel *T. muris*. However certain mouse strains, and indeed some individuals within particular mouse strains, are unable to mount a protective immune response and harbour long term chronic infections. This unique model thus presents an opportunity to examine the immune events underlying both resistance to infection and persistent infection within the same host species, and in some cases, the same host strain.

In 1967, infection of the outbred Schofield strain of mouse with the intestinal nematode parasite *Trichuris muris* revealed variation in the ability of individuals to eliminate the parasite (Wakelin, 1967). Thus, although the majority of mice were resistant to infection, 25–30% of mice were unable to expel the parasite and harboured sexually mature infections at day 35 post-infection (p.i.). These non-resistant mice were equally susceptible to secondary infections. The observation that some individual mice were unable to expel a *T. muris* infection was reiterated in 1970 using a broader profile of outbred mouse strains, with again small percentages of individuals being 'unresponsive' to infection (Wakelin, 1970). The 'resistant versus non-resistant' theme was continued in 1975 using, this time, eight different inbred mouse strains (Wakelin, 1975). This study by Wakelin clearly illustrated that the kinetics of worm expulsion were strain dependent and also revealed the inability of some individuals of the DBA/2 strain to expel the parasite. Later still, mouse strains wholly susceptible to *T. muris* infection were discovered (Else & Wakelin, 1988). These fundamental observations have had a profound impact on the study of immunity to intestinal nematode parasites, providing us with arguably the most powerful of laboratory models with which to investigate immunity to these types of infections. Thus, the existence of both individuals within the same mouse strain and different strains of mouse, which harbour either acute or chronic infections, allows a detailed dissection of the immune responses underlying resistance and susceptibility to infection.

A critical role for the CD4+ T cell in resistance to a parasite closely related to *T. muris*, *Trichinella spiralis*, had already been identified (Grencis *et al.*, 1985) when the ability to differentiate between types of CD4+ T cells on the basis of their cytokine production was discovered (Mosmann & Coffman, 1989). Thus, Th1 cells secrete interferon-gamma (IFN-γ), lymphotoxin and interleukin-2 (IL-2), with IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 being produced by Th2 cells. The existence of at least two helper T cells subsets, Th1 and Th2, and indeed the more recently established existence of T regulatory cells (Shevach, 2000; Trinchieri, 2001), provided an important framework on which to build an understanding of the cell regulatory mechanisms underlying acute and persistent infections. When the Th1/Th2 paradigm was applied to *T. muris* infection it became clear that the type of T helper cell that dominated in the mesenteric lymph node (MLN) draining the site of infection dictated the outcome of infection. Thus, mouse strains, or individuals within a mouse strain, which expelled *T. muris* mounted a strong Th2 response, characterized originally by the presence of IL-4, IL-5 and IL-9 in the relative absence of IFN-γ. In contrast, susceptible mouse strains, or individuals within a mouse strain, were Th1 dominated, with cells in the MLN making large amounts of IFN-γ (Else & Grencis, 1991;

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Else *et al.*, 1992, 1993). Clearly susceptible mice were not 'unresponsive' to infection, they were actually making a very profound immune response, but the type of response was inappropriate for worm resolution.

Following the demonstration that CD4+ T cells were essential in the protective immune response to *T. muris* (Koyama *et al.*, 1995) studies using cytokine knockout mice, *in vivo* cytokine depletions and the administration of cytokines to resistant or susceptible mice confirmed the importance of the Th1 and Th2 cytokines in determining the fate of a *Trichuris* infection (Else *et al.*, 1994; Bancroft *et al.*, 1997, 1998; Faulkner *et al.*, 1997). These types of study also lead to the identification of other key cytokines. Thus, as our knowledge of the cytokines important in resistance and susceptibility to *T. muris* infection grew, so our model of the cellular mechanisms that precipitated acute or chronic infection grew and expanded to accommodate an increasing list of pivotal cytokines. Our model has therefore moved on from one revolving primarily around IL-4 and IFN-γ. Resistance to infection involves not only IL-4, but also IL-9, IL-10, IL-13 and TNF-α (Bancroft *et al.*, 1998; Artis *et al.*, 1999; Richard *et al.*, 2000; Schopf *et al.*, 2002). Interestingly, IL-10 appears to play a dual role during infection, promoting resistance but also protecting the host from the immunopathology produced during infection (Schopf *et al.*, 2002). Very recently, IL-1α and IL-1β have also been identified as key players in protective immunity to *T. muris*. (H. Helmby & R.K. Grencis, unpublished). Absence of any of these cytokines impairs the protective immune response. Likewise our knowledge of the factors driving a susceptible phenotype now includes IL-12 and IL-18 as well as IFN-γ (Bancroft *et al.*, 1997; Helmby *et al.*, 2001).

Interestingly, the inclusion of cytokines with innate cellular sources allows us to speculate how the innate immune response influences the quality of the adaptive immune response (Medzhitov & Janeway, 2000, 2002). Thus the combination of pattern recognition receptors on innate cells of susceptible mice may differ in quantity or quality to those on the innate cells of resistant mice, allowing differences in cellular triggering and cytokine production. A reduced efficiency of innate cells of susceptible mice to respond to mucosally delivered antigens with the production of Th2 promoting cytokines could thus dictate the outcome of infection. In this context, increased or decreased risk of disease due to differences in innate immune responses are issues that are becoming more apparent with the discovery of polymorphisms in human TLR4 and its coreceptor CD14 (Baldini *et al.*, 1999; Arbour *et al.*, 2000).

Despite a good understanding of the cellular mechanisms that control resistance and susceptibility to infection, the Th2 controlled effector mechanims that culminate in expulsion of *T. muris* remain unidentified. Helminth infections induce strong mast cell, eosinophil and antibody responses. However, these candidate Th2 controlled effector mechanisms have been difficult to implicate in the expulsion of primary *Trichuris* infections. Worms can be expelled from the gut in the absence of mast cells, in the absence of eosinophils and in the absence of parasite specific antibody (Else & Grencis, 1996; Betts & Else, 1999; E.J. Rice & K.J. Else, unpublished). Recent data has, however, implicated the eosinophil in efficient worm expulsion, in that mice with delayed eosinophil recruitment to the gut exhibit a small but significant delay in worm expulsion (H. Dixon, J. Christie, M.E. Rothenberg & K.J. Else, unpublished). Recruitment of CD4+ T cells to the gut associated lymphatic tissue (GALT) appears to be important in the effector mechanism. Thus, severe combined immunodeficiency mice given primed CD4+ T cells become resistant to infection unless T cell recruitment to the gut is blocked using anti-β7 and anti-MAdCAM-1 antibodies (Else & Grencis, 1996; Betts *et al.*, 2000). In the absence of other obvious effector mechanisms, a strong candidate remains the CD4+ intraepithelial lymphocyte (IEL) which was suggested as an effector cell in 1983 (Lee *et al.*, 1983). *Trichuris muris*, within its intraepithelial niche, may be particularly susceptible to this sort of effector response, perhaps mediated by CD4+ IEL-derived Th2-type cytokines altering epithelial cell turnover. In this context, the involvement of TNF-α in the resistance mechanism may be to upregulate receptors for Th2 cytokines in the intestinal microenvironment, as suggested by Artis *et al.* (1999).

With the observations that local recruitment of leukocytes to the GALT is important in resistance to infection, attention is turning towards differences in the ability of resistant and susceptible mice to recruit the appropriate effector cells, once they have been induced. In this regard, the response of the gut epithelial cell to the insult of initial invasion by the parasite may play an important role. Epithelial cells are known to express many members of the chemotactic cytokine family, the chemokines, and interestingly, Th1, Th2 and IELs respond differently to different chemokines (Bonecchi *et al.*, 1998; Siveke & Hamann, 1998; Kunkel *et al.*, 2000). Certainly the CCL2 (monocyte chemoattractant protein-1 (MCP-1)) knockout mouse is very susceptible to *T. muris* infection despite the deficiency being expressed on a resistant background (M.L. deSchoomeester, M.C. Little, B.J. Rollins & K.J. Else, in preparation). Whether susceptibility is due to alterations in the recruitment of antigen presenting cells to the mesenteric lymph node, thus favouring a Th1 promoting environment is unclear as yet, but certainly macrophage recruitment to the infected gut is significantly reduced in the absence of CCL2.

Our understanding of the mechanisms of immunity to *T. muris* infection has evolved over recent years, with the existence of resistant and susceptible strains of mouse still pivotal in the research undertaken. Tools available to undertake this research have also developed, allowing us to address questions which were perhaps previously unavailable. In this context, the microarray technology has allowed us to look for differences in gene expression profiles between infected resistant and susceptible mice at the induction site, the MLN and the effector site, the gut, and to identify changes in the expression of genes common to particular pathways. From these sorts of studies novel candidate genes may be implicated in resistance to *T. muris*, and our preliminary data here suggest an important role for retinol binding protein in the generation of a Th2 type response (R. Datta, A.M. Brass & K.J. Else, unpublished), supported by a strong

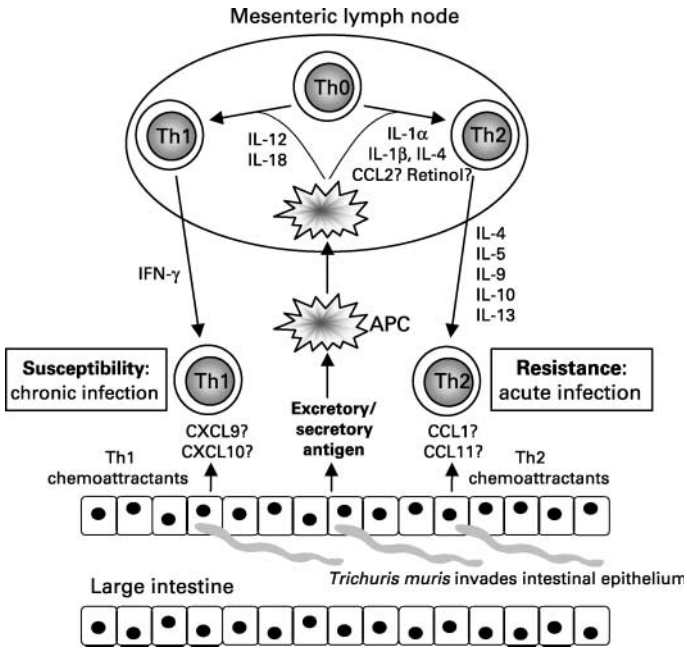

Fig. 1. Antigen drains to the mesenteric lymph node where T cells are polarized towards either Th1 or Th2 cells, according to the balance of multiple signals in the microenvironment. In the presence of IL-1α, IL-1β, IL-4 and other contributing factors, such as perhaps retinol and CCL2, T cells are driven towards Th2. In contrast, in the presence of IL-12, IL-18 and /or IFN-γ, the development of Th1 cells is promoted. At the level of the gut, epithelial cells respond to the insult of infection by upregulating chemokines. The portfolio of chemokines produced dictates which cell types are recruited locally, culminating in either resistance or susceptibility to infection. (APC, antigen presenting cell; CCL1, TCA-3; CCL11, eotaxin; CXCL9, Mig; CXCL10, IP-10).

literature illustrating that retinol is key in Th2 cell development (Carmen *et al.*, 1992; Stephensen *et al.*, 2002).

Thus, within the infected host, the balance between multiple signals shapes the quality of the adaptive immune response (fig. 1). The early innate immune response to infection may result in up-regulation of IL-1α, IL-1β or IL-4 from unidentified cellular sources, or IL-12 and/or IL-18, favouring Th2 or Th1 polarization within the MLN, respectively. In addition, other factors, including for instance the presence or absence of retinol and CCL2, may join together in providing a Th2 or Th1 inducing environment. At the effector site, the gut mucosa, early responses to parasite invasion by epithelial cells may be reflected in the up regulation of chemokines that recruit effector cells to the site of infection. The profile of chemokines provoked dictates whether appropriate or inappropriate cell types are drawn into the gut. The recruitment of CD4+ T cells secreting Th2 cytokines to the intraepithelial compartment may result in worm expulsion with direct effects of these cytokines on epithelial cell turnover representing an attractive effector mechanism.

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