

Interferon gamma is a key cytokine in lung phase immunity to schistosomes but what is its precise role?

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Abstract

Vaccination of mice with radiation-attenuated cercariae of *Schistosoma mansoni* induces a high level of protection against challenge with normal larvae. The immune effector mechanism, which operates in the lungs, is a cell-mediated delayed-type hypersensitivity response and involves the formation of a tight focus of mononuclear cells around embolised larvae. CD4⁺ T cells with Th1 characteristics are a major component of the infiltrate. They secrete abundant interferon gamma (IFN γ) upon antigen stimulation in vitro, whilst in vivo neutralisation of the cytokine results in 90% abrogation of immunity. IFN γ can induce a large number of genes and an attempt has been made to identify the ones which are essential components of the effector mechanism. Inducible nitric oxide synthase (iNOS) is such a candidate and nitric oxide (NO) is produced by cultures of airway leucocytes from the lungs of vaccinated mice post-challenge. However, the continued resistance of mice with a disrupted iNOS gene indicates that NO has only a minor role in the protective response. Mice with a disrupted IFN γ receptor gene have been used to dissect the role of the cytokine. After vaccination and challenge, CD4⁺ T cells from the pulmonary interstitium have reduced levels of ICAM-1 and LFA-1 expression, compared to wild-type animals, which coincides with a reduced cohesiveness of foci. However, immunity is not significantly impaired in mice with a disrupted ICAM-1 gene, and focus formation is normal. Similarly, a role has not been found for CD2/CD48 interactions in cell aggregation. Possible IFN γ -inducible molecules yet to be fully investigated include other ligand-receptor pairs, chemokines, and tumour necrosis factor α .

Key words

- Interferon gamma
- *Schistosoma mansoni*
- Lung phase immunity
- Nitric oxide
- Adhesion molecule

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Radiation-attenuated cercariae of *Schistosoma mansoni* elicit a high level of protective immunity against a challenge with normal cercariae. In C57BL/6 mice it ranges from 60-70% after a single vaccination, and in primates such as the baboon and vervet monkey it is >50% after 3 exposures (1). It has recently been shown that administration of recombinant IL-12 to mice around the

time of vaccination will boost that immunity to approximately 90%, with some animals having a zero worm burden (2). Indeed, the radiation-attenuated vaccine currently provides the best paradigm for the development of a human schistosome vaccine. With this goal in mind we have undertaken a detailed analysis of the mechanisms underlying both the induction and effector phases of the mu-

rine immune response to vaccinating and challenge parasites, respectively.

In mice, optimally attenuated larvae undergo a truncated migration which primes the immune system more potently than normal larvae. A proportion remains at the skin infection site, others enter and persist in the skin draining lymph nodes, whilst the remainder travel only as far as the lungs. The larvae which lodge in the lymph nodes trigger an intense proliferation in the T cell compartment, greater than elicited by an equivalent number of normal larvae. The cytokine profile secreted by antigen-stimulated T cell cultures over the first 7-10 days is a mixed Th1/Th2 (or Th0) response, with interferon gamma (IFN γ), interleukin (IL)-4, IL-5, and IL-10 all present (3). From day 10 onwards, the response polarises in the Th1 direction with approximately 25 times more IFN γ produced by T cells from mice exposed to attenuated than to normal parasites. Coincident with the T cell proliferation in the lymph nodes, delayed-type hypersensitivity (DTH)-mediating cells appear in the circulation, peaking around day 17 postexposure, before declining to background levels by day 35 (4).

Those attenuated larvae which migrate as far as the lungs also appear crucial to the induction of a protective response. Their arrival in the pulmonary vasculature stimulates recruitment of leucocytes to the pulmonary parenchyma and airways. The latter cells can be recovered by broncho-alveolar lavage and resolved by flow cytometry into granulocytes, lymphocytes and macrophages on the basis of their log 90° and forward light scatter properties (5). In contrast to the naive mouse where macrophages predominate, granulocytes and lymphocytes are abundant in the airways of vaccinated animals, with infiltration peaking between 17 and 21 days. The granulocytes, largely eosinophils, are rapidly cleared whilst the lymphocytes persist at least to 10 weeks. CD4⁺ T cells predominate in the lymphocyte population

and they secrete large amounts of IFN γ when stimulated with antigen *in vitro* (6) but they are reluctant to proliferate *in vitro*. They have the phenotypic features of short-term effector/memory cells (7) staining strongly for CD44 but are negative for the B isoform of CD45RB, which is present on naive T cells. The end result of vaccination is that by 35 days, the murine host has generated a population of schistosome-specific CD4⁺ T cells with Th1 characteristics and these cells are particularly numerous in the lungs. We have suggested that the recruited T cell population arms that organ against incoming normal challenge larvae.

Tracking of ⁷⁵selenomethionine-labelled parasites in primed mice has revealed that the major site of challenge elimination is the lungs (8). The immune effector mechanism has the features of a focal DTH response, with CD4⁺ and CD8⁺ T cells plus macrophages aggregating around an embolised larva (9). This reaction differs markedly from the one containing numerous epithelioid macrophages and eosinophils that develops around an embolised schistosome egg. The CD4⁺ T cells are key components of the response and their *in vivo* ablation, by administration of anti-CD4 monoclonal antibody (mAb) to mice, abrogates immunity (10). We have studied the kinetics of focus formation after administration of lung schistosomula, recovered from a donor animal, to embolise in the pulmonary vasculature of a primed mouse (11). The first cells aggregating around parasites are visible in perivascular locations by 24 h. Infiltration intensifies by 48 h, but the greatest influx occurs between 48 and 96 h and foci are at maximum size by 8 days. It is important to realise that a schistosomulum arriving in the lungs from the skin must first elongate in order to migrate along capillaries to reach the venous compartment. The 3-4-day duration of this developmental process coincides almost exactly with the time needed for focus formation, and hence may explain why

some schistosomula can evade the effector response and exit the lungs to reach the systemic circulation. Indeed, schistosomula may return to the lungs several times in the course of their migratory circuits around the vascular system, but on second and subsequent passages they will already be elongated and able to traverse the capillaries more rapidly.

Production of IFN γ is crucial to focus formation and protection. Administration of anti-IFN γ mAb to vaccinated mice between 4 and 16 days after challenge abrogates protection by 90% (12) and also results in the production of loose and disseminated cellular infiltrates in the lung. It is therefore pertinent to ask what precise role IFN γ might play in the effector response. Since CD4⁺ T cells are a prerequisite for the immune effector response, the relevant antigens must be released by the embolised larvae for processing and presentation by accessory cells to bring about their activation. (As yet we know little about this initiating event.) IFN γ can induce a large number of genes and so it is a difficult task to pinpoint the ones most relevant to the effector process. For the purposes of this review those genes can be divided into two broad categories. The first involves the enzymes that control production of cytotoxic agents such as nitric oxide (NO) or superoxide which could directly kill the parasites. The second category covers those activities concerned with the generation of inflammation.

Newly transformed schistosomula are very susceptible to cytotoxic killing mechanisms *in vitro* but paradoxically lung stage larvae are refractory. We have investigated the potential role of NO as an agent in the pulmonary effector response *in vitro* and *in vivo* (13). Both antigen-stimulated and spontaneous production of NO by cultures of airway leucocytes from vaccinated and challenged mice are greater than those from challenged control animals. RT-PCR analysis of mRNA for inducible nitric oxide synthase

(iNOS) has also revealed its upregulation *in vivo*, 96 h after intravenous challenge of vaccinated mice with lung schistosomula, coincident with focus formation.

We tested the hypothesis that NO production influences the protective immunity elicited by vaccination, using mice with a targeted disruption of the iNOS gene. In two vaccination experiments, the inability to induce NO production resulted in a reduction in protection from 68 to 48% (an abrogation of approximately 30%). However, the situation was more complicated due to the fact that both test and control gene-disrupted mice had higher worm burdens than their heterozygous littermates. Application of ANOVA to the data detected no significant effect on worm burden attributable to iNOS disruption. We therefore concluded that NO does not play a major role in worm elimination in this model. One contributory factor to the situation may be the ability of haemoglobin to scavenge NO and convert it to nitrate. Thus, when mouse erythrocytes are added to *in vitro* larvicidal assays, they completely abrogate the killing of newly transformed schistosomula. Since schistosomula migrate from the skin to the portal tract within the bloodstream, they will at all times be surrounded by erythrocytes and it is unlikely that they will be exposed to cytotoxic concentrations of NO.

The minor role indicated for NO production in the pulmonary effector response provides confirmation for earlier observations made on the viability of challenge schistosomula in the lungs (14). Tracking studies have revealed that by day 17 migration of challenge parasites in vaccinated mice has virtually ceased, yet many schistosomula remain in the lungs and will never mature. Nevertheless, if these parasites are recovered and injected into the hepatic portal system of a naive mouse, approximately 75% will mature, virtually the same as for parasites recovered from the lungs of challenge controls. These data provide strong evidence

that schistosomula are simply trapped by the inflammatory infiltrate. In other words, the pulmonary effector mechanism operates by blocking migration rather than by direct cytotoxic killing.

If blocked migration is the key to understanding the mechanism(s) of protection, then what role does IFN γ play in this process? In this context, we have used mice with a disrupted IFN γ receptor (IFN γ R^{-/-}) gene; these animals synthesise the cytokine but are unable to transduce its signals at the cell surface. They develop approximately 20% immunity to challenge after a single vaccination, compared to the 10% protection seen after neutralisation of IFN γ in C57BL/6 mice. It might be anticipated that disruption of IFN γ signalling pathways would reduce pulmonary inflammation. In fact, it increased three-fold over the wild-type animals, with much larger numbers of eosinophils present. The pulmonary CD4⁺ T cells have Th2 characteristics, making abundant IL-4, IL-5 and IL-10. As with IFN γ neutralisation, histopathological observations reveal a very loose and generalised infiltrate, compared to the tightly circumscribed foci found around challenge parasites in intact vaccinated C57BL/6 mice.

A potential explanation for the lack of cohesion in the foci from IFN γ R^{-/-} mice is that one function of IFN γ is to upregulate adhesion molecules, such as LFA-1 and ICAM-1, necessary for homotypic and heterotypic adhesion. To investigate this possibility, we recovered CD4⁺ T cells from the pulmonary parenchyma 14 days after percutaneous challenge, when foci are maximal in size, and phenotyped them for expression of the ligand-receptor pair. In C57BL/6 mice 70% of the cells were positive for ICAM-1 with only 26% in IFN γ R^{-/-} mice (Coulson PS, unpublished data). There was a similar but less dramatic pattern for LFA-1 staining with a 40% reduction in the mean intensity of expression in the latter group, compared to the former. It thus seemed likely that

reduced ICAM-1/LFA-1 interactions were responsible for the loss of cohesiveness in foci, and hence reduced resistance. We therefore investigated the protective ability of the attenuated vaccine in C57BL/6 mice with a disrupted ICAM-1 gene. In three separate experiments, we found that protection was reduced by a mean of 15%, compared to the level in intact wild-type mice (Coulson PS, unpublished data). Furthermore histopathological examination of the lungs from the gene-disrupted mice revealed no loss of focal integrity. We therefore concluded that the absence of ICAM-1 on leucocytes within foci had little effect on protective immunity.

CD2 and CD48 (the murine equivalent of LFA-3) are a second ligand-receptor pair involved in intercellular adhesion. We therefore undertook a phenotypic analysis of CD2 and CD48 expression on the CD4⁺ T cells recovered from the pulmonary interstitial compartment of vaccinated mice at 14 days postchallenge, in comparison with cells from the lymph nodes (Coulson PS, unpublished data). Both ligands were expressed at a higher level in the pulmonary than the lymph node T cell populations in wild-type mice. However, there was no reduction in expression on the T cells from the lungs of IFN γ R^{-/-} mice. Indeed, there appeared to be an increase in CD48 expression compared to wild-type animals. It therefore seems unlikely that CD2/CD48 interactions are a key element regulated by IFN γ to control focus integrity.

Our data have highlighted the central role of IFN γ production by CD4⁺ T cells as the likely primary regulator of effector focus formation. However, in spite of promising leads, we have yet to define the subsequent cascade of events in this model of acquired immunity to schistosomes. What options remain to be explored in the quest to understand the formation of the pulmonary effector focus? There are other known ligand-receptor pairs such as CD40/gp39 which could play a role. It also seems plausible that chemokines would be involved in the pro-

cess of cell accumulation, and the induction of such molecules by IFN γ has been documented (15). Finally, IFN γ is a potent stimulator of TNF α production by macrophages,

and the potential involvement of this second proinflammatory cytokine requires investigation.

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