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T CELL DERIVED CYTOKINES IN LUNG-PHASE IMMUNITY TO *SCHISTOSOMA MANSONI*

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In C57Bl/6 strain mice vaccinated with radiation-attenuated cercariae of Schistosoma mansoni *immune elimination of challenge parasites occurs in the lungs. Leucocytes were recovered from the lungs of such mice by bronchoalveolar lavage and cultured* in vitro *with larval antigen; the profile of cytokines released was then analyzed. From 14 days after vaccination, BAL cultures contained infiltrating lymphocytes which produced abundant quantities of IFN-g and IL-3. Challenge of vaccinated mice resulted in a second influx of IFN-g and IL-3 – producing cells, earlier than after vaccination or in the appropriate controls. Ablation studies revealed that $CD4^+$ T cells were the source of IFN-g. The timing of cytokine production after vaccination, and challenge was coincident with the phases of macrophage activation previously reported. At no time could lymphocytes in BAL cultures be stimulated to proliferate with either larval Ag or mitogen, in contrast to splenocytes from the same mice. Furthermore, T cell growth factor activity was not detected in BAL cultures stimulated with Ag. We suggest that the lymphocytes recruited to the lungs are memory/effector cells. When Ag released from challenge schistosomula is presented to these cells, they respond by secreting cytokines which mediate the formation of cellular aggregates around the parasites, blocking their onward migration.*

Key words: *Schistosoma mansoni* – immunity – T cell cytokines – lung-phase

A single exposure of C57Bl/6 mice to 500 cercariae of *Schistosoma mansoni*, attenuated by 20Krad of gamma radiation from a ^{60}Co source, induces 60-70% protection against a challenge with 200 normal cercariae. Numerous studies have demonstrated that protection is dependent on specific acquired immune mechanisms (Wilson & Coulson, 1989; James & Sher, 1990) rather than pathology (Wilson, 1990). In once-vaccinated animals, immunity is largely abrogated by administration of anti-$CD4^+$ antibodies around the time of challenge, indicating that the effector response is mediated by T helper lymphocytes (Vignali et al., 1989).

The site of challenge elimination in previously vaccinated mice has been determined using autoradiography of compressed tissues to detect the presence of ^{75}Se-labelled parasites (Georgi, 1982; Dean et al., 1984). Provided that all host tissues are processed, it is possible to build up a balance sheet of parasite loss with time from which potential sites of death can be identified (Wilson et al., 1986). Fourteen days post-challenge when elimination is just beginning, parasites are distributed in the ratio 1 skin: 5.8 lungs: 0.8 systemic organs. At day 21 when migration is complete, approximately half of the penetrating parasites are still detectable in organs other than the liver, particularly the lungs. They will never mature and so we have concluded that in this particular strain of mouse the bulk of immune-mediated elimination occurs in the lungs.

An important question we have tried to answer is why radiation-attenuated parasites should induce a protective response when an equal number of normal parasites do not. We have identified significant differences in the pattern of migration which may hold the key (Mountford et al., 1988). Approximately 15% of normal schistosomula leave the skin infection site via the lymphatics and pass rapidly through any lymph nodes encountered to reach the blood vascular system. A similar proportion of attenuated parasites take this route but, in contrast, become trapped in the nodes and persist there for up to 14 days. Studies both *in vitro* and *in vivo* (Constant, et al., 1990; Pemberton et al., 1991) have revealed that the prolonged residence of the larvae stimulates an intense proliferation of lymphocytes in the

nodes. This response has been characterised *in vivo* by measuring the incorporation of the thymidine analogue bromo-deoxyuridine into dividing cells (Constant & Wilson, 1991). When the technique was combined with identification of lymphocytes by surface phenotype, the proliferative response in axillary lymph nodes draining the exposure site was found to occur predominantly in the T cell compartment. The total number of B cells in the nodes increased dramatically, but apparently by a process of retention, rather than proliferation.

Proliferation *in vitro* following stimulation with a soluble antigen preparation derived from 18th schistosomula, has also been investigated (Pemberton et al., 1991). The response peaks early at around 5 days post-exposure before declining to low levels by day 21. In this respect it differs significantly from the pattern observed *in vivo* (Constant & Wilson, 1991) which indicates a peak of cell division around day 14. However, interpretation is complicated by the retention of B lymphocytes in the nodes which has the effect of diluting the T cells in a sample ex vivo, to different degrees, at different times post-infection. Comparison of responses elicited by exposure to normal parasites has revealed that initially they are identical to those induced by attenuated larvae. This presumably indicates that the early responses in the nodes are predominantly to cercarial released products. These should be similar whether originating from normal or attenuated larvae, since both can penetrate the skin equally well. At later times responses induced by attenuated larvae are stronger, reflecting their persistence in the nodes, compared to normal larvae.

T helper lymphocytes have been divided into two subsets on the basis of the patterns of cytokine released by cloned cell lines *in vitro*. The Th1 subset is characterised by IL-2 and Interferon gamma (IFNg) production, and Th2 cells by IL-4 and IL-5 whilst IL-3 is a product of both (Mosmann & Coffman, 1989). We have examined the profile of cytokines produced by cultures of lymph node cells to establish which subset is induced by vaccination (Pemberton et al., 1991). In the early stages of the response (day 5-6) we detected production of IL-2, IL-3, IL-4 and IFNg. At later times (day 21) IL-3 and IFNg were predominant, IL-2 production was low and IL-4 undetectable. The abundant production of IFNg implies the induction of a Th1 response. Some idea of the efficacy of vaccination can be obtained if the potential IFNg production of the axillary lymph nodes of mice exposed to attenuated or normal parasites is compared at day 21. Taking into account the total cell content of the nodes, vaccinated mice have 24x times the potential for IFNg production as animals exposed to normal cercariae.

Following induction of the primary immune response in the skin-draining lymph nodes, lymphocytes capable of mediating a classical footpad DTH response appear in the circulation (Ratcliffe & Wilson, 1991a). They peak around day 17 before declining to background levels by day 35. Their presence in the circulation over this period may be crucial to a second component responsible for the success of the attenuated vaccine.

A proportion of attenuated parasites migrate from the skin to the lungs, starting on day 4 and reaching a plateau by day 10 (Mountford et al., 1988). Unlike normal schistosomula, which are able to negotiate the pulmonary capillaries and continue their migration, optimally irradiated parasites go no further than the lungs where they persist at least to day 21 (Mastin et al., 1985). An inflammatory response is triggered by their presence, and recruits leucocytes from the circulation to the pulmonary parenchyma and airways. Studies with ^{51}Cr-labelled splenocytes have shown that the recruitment occurs between 10 & 20 days post-vaccination (Ratcliffe & Wilson, 1991b). It is most intense around day 17 when DTH-mediating lymphocytes are at their peak in the circulation. It thus seems reasonable to assume that, even if lymphocytes are recruited randomly, a significant proportion will be of this category.

Cells representative of the pulmonary infiltrate can be recovered from the lungs by broncho-alveolar lavage (BAL) for detailed study. Analysis by flow cytometry using forward and log 90° light scatter properties resolves the BAL population into three distinct components: lymphocytes, polymorphonuclear cells, and macrophages (Aitken et al., 1988). In contrast BAL cells recovered from naive mice are almost entirely macrophages. Peak numbers of infiltrating cells are found approximately 21 days post-vaccination. The polymorphonuclear cells, largely eosinophils, are rapidly cleared but the lymphocytes persist at

elevated levels at least to 10 weeks post-vaccination. The majority are $CD4^+$ T helper cells, with a minor component of $CD8^+$ cytotoxic T cells, and less than 5% B lymphocytes. We have suggested that the function of there recruited T cells is to "arm" the lungs against the arrival of normal challenge schistosomula.

The pulmonary T cells have some unusual properties and differ from splenocytes recovered from the same mouse in the kinetics of their response to antigen (Smythies et al., 1992a). Using IFNg production by cell cultures as the index of activity, we have shown that day 21 BAL cells need less antigen and respond much more quickly than spleen cells. Indeed, at times of peak reactivity, T lymphocytes in the BAL cultures will produce IFNg in the absence of added antigen. Either they were activated by *in vivo* conditions before lavage, or the pulmonary macrophages recovered with them expressed schistosome antigen which had been released in the lungs.

The antigen-stimulated production of IFNg by BAL cell cultures is not uniform with time after vaccination. It begins to increase between days 7 and 14 reaching a peak at day 21, before falling back virtually to zero by day 35. This decline occurs is spite of numerous lymphocytes being present in the cultures. Additionally, we have detected production of IL-3 after vaccination with a pattern similar to that for IFNg. The secretion of these two cytokines points to Th1 cells as the main constituent of the pulmonary infiltrate. However, the situation is complicated by the failure of the BAL T cells to proliferate in response to both antigen or mitogen. Additionally, they fail to secrete T cell growth factor (TCGF = IL-2 + IL4) when stimulated with antigen. In all these respects they are typical of memory/effector cells described in other experimental systems (Budd et al., 1987; Sanders et al., 1988).

When normal challenge schistosomula reach the lungs of previously vaccinated mice, they trigger an anamnestic inflammatory response which leads ultimately to their elimination. A newly arrived parasite, lodged in a pulmonary blood vessel, quickly attracts a mononuclear cell infiltrate comprising lymphocytes and macrophages (Crabtree & Wilson, 1986). Many of the cells are insinuated between the vascular endothelium and alveolar epithelium in proximity to the parasite. By 17 days post-challenge, a tight focus of cells has built up, surrounding the parasite and impeding its further progress. Immunophenotyping on cryostat sections has revealed these cells to be $CD4^+$ T helper lymphocytes, macrophages, and a smaller number of $CD8^+$ T cells (Kambara & Wilson, 1990). Each parasite is seen by the immune system as a unique event and focus formation may destroy the vascular highway along which it was migrating, deflecting it into an alveolus. Surprisingly the parasite appears, morphologically at least, to be unaffected by the inflammation (Crabtree & Wilson, 1986). Schistosomula in the lungs of vaccinated mice at day 17, will not mature if left *in situ* but, if recovered by mincing and incubation, and injected into the portal vein of naive animals, they are able to do so (Coulson & Wilson, 1988). These observations imply that the effector mechanism of immunity functions by blocking migration, rather than by direct cytotoxic killing.

Cellular events occurring after challenge have been characterised in several ways. There is a rapid recall of DTH-mediating cells to the circulation. Peak levels are attained by seven days, although the magnitude of the response, measured by footpad assay, is no greater than after vaccination (Ratcliffe & Wilson, 1991a). A second phase of cellular infiltration can be detected by BAL (Smythies et al., 1992a) but not by the accumulation of ^{51}Cr-labelled splenocytes in the lungs (Ratcliffe & Wilson, 1991b). In this second phase which peaks on day 14, eosinophils are not prominent, but T lymphocyte numbers increase significantly (Smythies et al., 1992a). IFNg production by BAL cultures rises more rapidly after challenge than after vaccination, reaching a peak at day 14, before declining. The period of production coincides exactly with that of challenge parasite elimination in the lungs. The earlier onset of cytokine production implies an anamnestic response and lends support to the hypothesis that recruited lymphocytes do indeed arm the lungs, permitting a rapid response when challenge parasites arrive.

The most likely role of IFNg is to activate pulmonary macrophages. The ability of these cells, recovered by BAL from vaccinated and challenged mice, to mount an oxidative burst increases rapidly between days 7 and 14 (Menson & Wilson, 1989). Macrophages from challenge control mice also show an increased oxidative burst, but delayed by about seven days, too late to affect the migration of

schistosomula through the lungs. The central role of IFNg in the immune effector mechanism has been confirmed in ablation experiments using monoclonal antibodies (MAB) (Sher et al., 1990; Smythies et al., 1992b). In our experiments, administration of anti-IFNg MAB to control mice between day 4 and 16 post-challenge had no effect on their worm burden compared to similar untreated animals. In contrast, when the MAB was administered to vaccinated and challenged animals, the worm burden was greatly increased compared to that in a similar untreated group. Indeed it was not significantly different to that in the challenge controls, the increase amounting to a reduction in immunity of 80%.

Paradoxically, in the vaccinated and challenged animals receiving MAB, the level of pulmonary eosinophilia was greatly elevated at day 14, compared to the parallel untreated group (Smythies et al., 1992b). This observation suggests that there is an inverse relationship between IFNg production and eosinophilia. One potential explanation is that the pulmonary macrophages, activated by IFNg, are responsible for the rapid clearance of eosinophils from the airways. This suggestion was first made by Crabtree & Wilson (1986) on the basis of ultrastructural evidence. They observed alveolar macrophages, in sections of lung tissue from vaccinated and challenged mice, in the act of phagocytosing eosinophils. The macrophages also contained paracrystalline inclusion bodies which it was speculated were the indigestible remains of eosinophil granular proteins.

Numerous questions remain to be answered about this model of specific acquired immunity to *S. mansoni* before the full potential of the mechanisms involved can be realised. The T lymphocytes which infiltrate the lungs, and provide the element of specificity in the effector response, merit further study. The antigens which mediate the protective response also urgently need to be identified and their genes cloned in a recombinant system to permit full characterisation. The manner in which these antigens, released by challenge schistosomula in the lungs are processed and presented to the pulmonary effector T cells needs to be established. These topics are the focus of our current research programme.

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