In vitro Lymphoproliferative Responses of *Trichostrongylus colubriformis* High and Low Responder Guinea Pigs to Worm Antigens (SPL3, SPA) and Ovalbumin

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ABSTRACT

*In vitro* lymphocyte responses of high responder (HR) and low responder (LR) guinea pigs from peripheral blood lymphocytes (PBL) to parasite antigens soluble protein third stage larvae (SPL3) and soluble protein adult stage (SPA), non-parasite antigen ovalbumin (OVA) were examined. There was substantial differences between HR and LR guinea pigs in the rate of acquisition of responsiveness to these Ags as well as differences in responsiveness to Ags derived from third stage larvae (SPL3) and adult worms (SPA). Overall, the results suggest both stronger and more rapid acquisition of responsiveness by HR animals and raise the possibility of the animals being able to preferentially respond to larval immunogen and thus acquire protective immunity more rapidly than LR. *Iran. Biomed. J* 2 15-20 (1998).

Keywords: *Trichostrongylus colubriformis*, soluble protein third stage larvae, soluble protein adult stage

INTRODUCTION

*In vitro* lymphoproliferative responses to Ag and mitogens have been used for many years to assess lymphocyte function and such assessments have been made in several host-parasite systems, including *T. colubriformis* and *Haemonchus contortus* (*H. contortus*) in sheep [1, 2]. The use of *in vitro* lymphocyte proliferation assay as a predictive test for assessing potential resistance of sheep to infection with gastrointestinal nematodes was successful with *H. contortus* [3] but results with *T. colubriformis* were not conclusive. Dawkins *et al.* [4] reported that cellular parameters did not differentiate between HR and LR lambs. On the other hand, Win-don and Dinneen [1] showed that sheep immune to *T. colubriformis*, had high *in vitro* lymphocyte responses to the *T. colubriformis* antigen preparation SPL3 when compared with susceptible animals. Immunodominant Ags from *T. colubriformis* were also recognized by T- lymphocytes from sheep immunized with excretory-secretory (ES) proteins of the parasite [5].

Although *in vitro* proliferation of lymphocytes from out bred guinea pigs in response to worm Ags has been studied [6], a comparison of these responses in HR and LR guinea pigs has not been made. It was decided to examine *in vitro* responses of lymphocytes from HR and LR guinea pigs to SPL3 and SPA. The purpose of this study was to determine whether the difference in susceptibility of these animals reflected differences in the activation of cells involved in expression of resistance, and in addition, whether SPL3 and SPA had different abilities to induce cell transformations. Because significant differences in responsiveness of HR and LR animals to *T. colubriformis* Ags were found, a similar experiment using OVA (a non-parasite Ag) to immunize guinea pigs was also performed.

MATERIALS AND METHODS

Experimental Design. Guinea pigs used in this work were derived from breeding stock with genetically determined resistance (HR) or susceptibility (LR) to *T. colubriformis* infection. These breeders were outbred and the product of a selective breeding program based on fecal egg counts.
Fecal Egg Counts. Geometric mean FEC of these HR and LR guinea pigs during challenge with *T. colubriformis* are depicted in Figure 1.
Throughout the period of observation, LR guinea pigs showed higher egg counts than HR animals (P<0.001). In LR, EPG reached a peak on day 23 (mean 2331, Fig. 1), thereafter fell to 1391 and 998 by days 25 and 28 respectively. HRs had a mean counts of 49 EPG on day 16 and no eggs we found in the feces after day 21.

**Fig. 1.** Eggs per gram faeces (geometric mean) of 4xHR and 4xLR guinea pigs following infection with 2000 infective larvae of *T. colubriformis*. high responder (HR), low responder (LR).

**Responses to Antigen** (Experiment A). Time courses of proliferative responses of PBL from HR and LR guinea pigs incubated with parasite Ags are shown in Figure 2. Poor blastogenic responses to SPL3 and to SPA were observed in both lines prior to experimental infection, but significant proliferative responses developed following infection. Blastogenic response to SPL3 was greater in HRs than in LRs following infection (P < 0.05). HR animals responded to SPL3 after primary infection, reaching mean cpm of 1972 v 106 (Figure 2). The peak response was detected on day 21 then fell by day 28. LR animals, however, showed a suppression in response to SPL3 on day 7 (P < 0.05) but positive responses developed as the infection progressed.

Proliferative responses to SPA in both lines did not show a significant change until day 7 of infection, and reached their peak on or after day 28. Split plot in time analysis revealed similar responses of both HR and LR animals to SPA following infection. However, the only between lines significant difference detected was on day 21 when HRs were more responsive than LRs (mean 16528 v 2852, Fig. 2).

Comparison of responses to the two Ags by means of split-plot in time analysis during primary infection showed similar responses in HRs to both Ags, whereas LRs were more responsive to SPA than to SPL3 (1511 v 383, P < 0.05 Fig. 3).

**Fig. 2.** Proliferative responses (geometric mean c.p.m.) of PBL from 4xHR and 4xLR guinea pigs to *T. colubriformis* antigens (SPL3, SPA) following infection. ■ high responder (HR), ▲ low responder (LR).
Non-parasite Antigen (Experiment B). In vitro lymphocyte proliferative responses of OVA-immunized guinea pigs to OVA are depicted in Fig. 4. Both lines responded to OVA following immunization \((P = 0.0017)\). Although there was a significant difference between the lines at all data points \((P = 0.04)\). Split plot in time analysis revealed that this difference was due to a difference on day 7, in which LR animals showed depressed responses to OVA. Both lines also peaked on 28 d after immunization (mean cpm of HR = 6888 and LR = 2315, Fig. 4).

**DISCUSSION**

This study demonstrates that the whole blood culture technique is satisfactory for lymphocyte proliferation assays in guinea pigs. Optimum stimulation time was generally 3 d at 37 °C plus 16-18 h methyl-\(^3\)H-thymidine pulse time. Zimmerman et al. [11] reported a cell density of \(8 \times 10^5\) cells per well to be optimum for ovine lymphocyte cultures using the whole blood technique. This density was within the ranges used in present study. Both lines revealed slight, but statistically insignificant increases in total leukocyte counts following infection that could be due to in vivo proliferation of lymphocytes to *T. colubriformis* infection.

The results demonstrated that PBL from both lines of guinea pigs acquired responsiveness to parasite Ags following infection with *T. colubriformis*. They also demonstrate substantial differences between HR and LR guinea pigs in the rate of acquisition of responsiveness to these Ags as well as differences in responsiveness to Ags derived from third stage larvae (SPL3) and adult worms (SPA). Further, responses to OVA were generally greater in HR animals and finally, in response to both parasite Ags and OVA, circulating lymphocytes from LR animals generally showed lower responsiveness when examined from 7 d after infection or immunization. However, it was previously reported (unpublished data) that parasite-infected and OVA-immunized guinea pigs revealed different isotype-specific antibody responses and significant difference between the lines observed following infection only. Overall, the results suggest both stronger and more rapid acquisition of responsiveness by HR animals and raise the possibility of the animals being able to preferentially respond to larval immunogen and thus acquire protective immunity more rapidly than LR.

This study supports the finding of Dobson and Soulsby [6] who reported peak proliferative responses of outbred guinea pigs to *T. colubriformis* Ags 25 d after infection. Of particular relevance is the observation of McClure et al. [12] that HR sheep responded to SPL3 stronger and earlier than LR sheep and that their responsiveness to SPL3 was stronger than their responsiveness to SPA. The different capacity of SPL3 and SPA to stimulate HR and LR lymphocytes, suggests that a variety of Ags in each of these preparations provoke immune responses in the host. Such Ags might be common between larvae and adults or there might be quantitative or qualitative differences between the Ags in the different parasite stages. For example, a 94
kDa glycoprotein from TcL3 [13], 18 kDa and 30 kDa from TcA [14, 15] and a 41 kDa from both TcL3 and TcA (16) have been reported to be capable of partially protecting hosts against T. colubriformis. Thus, SPL3 may contain a greater number of Ags with stronger abilities than those in SPA to elicit protective immune responses in guinea pigs. This may account for inverse correlation between FEC and increasing cell transformation by SPL3 (days 14 and 21) and SPA (day 21) where peak proliferation occurred at the time worm eggs disappeared from the feces of HR guinea pigs (Fig. 1).

Despite similar antibody responses of both lines to OVA (unpublished data), PBL from HR animals responded to OVA earlier than LR animals. Split plot in time analysis revealed that LRs exhibited significantly depressed responsiveness (cpm) on day 7. Faster responses to OVA in HRs (day 7 to 14) than in LRs (day 21 to 28) may be due to enhanced Ag presentation to T-cells of HRs. Lutje and Black [17] reported that OVA-specific in vitro responses of in vivo primed PBL were dependent on the presence of CD4+ T-cells to which OVA was presented in a MHC II restricted manner. Therefore, LRs might be genetically less capable of MHC II surface molecule expression following foreign Ag activation as they revealed lower cpm than HRs during immunization. Further, B-cells of guinea pigs contain considerably larger amounts of MHC II products than do T-cells [18] and they are capable of presenting Ag to primed T-cells in a MHC-restricted fashion [19, 20]. Neither macrophages nor dendritic cells can efficiently take up soluble Ags, but B cells are uniquely adapted to bind soluble molecules through their cell surface Ig. Both OVA and SPL3 are soluble proteins to which CD4+ T-cell responses require B cells as APC [21]. Thus, the relatively poor responses of LR guinea pigs to OVA might also be due to a lower frequency of B-cells [22] leading to a poorer ability in Ag presentation to CD4+ T-cells. This hypothesis was reinforced by higher (P=0.0005) ability of HRs to respond to PWM prior to and following immunization with OVA (unpublished data).

LR guinea pigs eventually develop resistance to T. colubriformis after termination of their primary infection. Their stronger response to SPA suggests that their less effective and delayed protective responses compared with HR animals may be due to their different profile of Ag recognition. Further, preliminary experiments on multiple-infected guinea pigs showed that LRs were more responsive to SPA than to SPL3 whereas HRs showed similar responses to both SPA and SPL3 (data not shown). Again, higher responses of HR animals to both Ags may be due to different immunodominant proteins recognized by HRs and LRs.

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REFERENCES


