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Modulation of the cellular immune response by a carbohydrate rich fraction from *Echinococcus granulosus* protoscoleces in infected or immunized *Balb/c* mice

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SUMMARY

Infection of Balb/c *mice with* Echinococcus granulosus *protoscoleces constitutes the model for secondary hydatid infection. The immune response of* Balb/c *mice infected with* E. granulosus *is characterized by secretion of antibodies specific for carbohydrate epitopes and production of type-2 cytokines. A role for glycoconjugates in the induction of type-2 responses has been suggested in other host–parasite systems. Although glycoconjugates are immunogenic in* E. granulosus *infection, the role of these molecules in the establishment of the type-2 response has never been analysed. In this study, a carbohydrate rich fraction ($E4^+$) from* E. granulosus *protoscoleces was obtained using the monoclonal antibody E492/G1 specific for the moiety Galα(1,4)-Gal which is widely represented in protoscoleces and other* E. granulosus *antigenic preparations. The results showed that $E4^+$ was immunogenic in* Balb/c *mice evoking an antibody response mainly directed against carbohydrate epitopes. In addition, splenocytes from $E4^+$-immunized mice showed suppressed proliferative responses to Con A and $E4^+$ induced IL-10 secretion by $E4^+$-primed and naive splenocytes. The fraction $E4^+$ also was immunogenic in infected mice during early infection. In this case also, splenocytes from infected mice as well as peritoneal cells from infected or naive mice, when stimulated* in vitro *with $E4^+$, secreted IL-10. Collectively, these results suggest that $E4^+$ may be involved in immunosuppression phenomena and, by stimulating IL-10 secretion, may contribute to the induction and sustaining of the type-2 cytokine response established in early experimental infection.*

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INTRODUCTION

Parasites usually produce long-lasting infections in immunologically competent hosts. Modulation of the host immune response has been proposed as a strategy to ensure parasite survival (Finkelman *et al*. 1991). Some parasites suppress the host immune response (Wakelin 1984) or induce type-1/type-2 cytokine responses which may determine the outcome of infection (Scott *et al*. 1989, Sher & Coffman 1992). It has been suggested that glycoconjugates are involved in both kinds of immunomodulation in some parasitic diseases such as alveolar hydatidosis and schistosomiasis (Velupillai & Harn 1994; Persat *et al*. 1996).

Infection with *Echinococcus granulosus* (the causative agent of hydatid disease) evokes cellular and humoral responses (Lightowlers & Gottstein 1995) but these are not able to eliminate the parasite. Glycoconjugates are quantitatively important in cysts and protoscoleces (McManus & Bryant 1995) and constitute major immunogens inducing an antibody response that has been well characterized (Ferragut & Nieto 1996, Míguez *et al*. 1996, Severi *et al*. 1997). In relation to the cellular response, *Balb/c* mice infected with *E. granulosus* protoscoleces produce a type-2 cytokine response during early infection (Dematteis *et al*. 1999). Thus, it is worthwhile analysing whether protoscolex glycoconjugates have a role in the induction of the type-2 cytokine response stimulated during early infection.

As a preliminary approach to this issue, we looked for immunomodulatory functions mediated by a previously described carbohydrate rich fraction ($E4^+$) (Baz *et al*. 1999) obtained from protoscoleces. For this purpose, we analysed the immunogenicity and cellular response elicited

by this fraction in infected or $E4^+$-inoculated *Balb/c* mice. The results suggest that $E4^+$ components may be involved in immunosuppression phenomena and may contribute to the early type-2 cytokine response stimulated by *E. granulosus* in *Balb/c* mice during early infection.

MATERIALS AND METHODS

Antigens

Protoscoleces were obtained by aseptic puncture of fertile bovine hydatid cysts according to Baz *et al*. (1995). Parasite viability was determined by eosin exclusion (Robinson & Arme 1985) and flame cell activity (Gurri 1963). Only those batches with over 95% viability were used for infection and antigen preparation. Protoscolex somatic antigen (PSA) was prepared according to Míguez *et al*. (1996) by ultrasound disruption of viable protoscoleces in the presence of EDTA and phenylmethylsulphonyl fluoride. Insoluble material was separated by centrifugation at 8000 ***g***. The supernatants were filtered through 0·22 μm sterile membranes and stored at -20°C. Protein and carbohydrate contents were determined as described below.

The monoclonal antibody E492/G1 (specific for Galα(1,4)Gal), coupled to Sepharose-CNBr, was used to purify a carbohydrate rich fraction ($E4^+$) from PSA according to Baz *et al*. (1999).

Protein and carbohydrate determinations

Protein content of antigenic preparations was determined by the bicinchoninic acid method (BCA Protein Assay Reagent, Pierce, IL, USA), according to the manufacturer's instructions, and carbohydrate content by the orcinol-sulphuric acid assay as previously described (White & Kennedy 1986).

Inoculations

A group of adult *Balb/c* mice ($n = 12$) was inoculated intraperitoneally with $E4^+$ (50 μg of carbohydrates per mouse) ($E4^+$-immunized mice) and a second group was inoculated with PSA (50 μg of carbohydrates per mouse) (PSA-immunized mice) in 200 μl of phosphate buffered saline (PBS). A third group (control mice) was inoculated with PBS (200 μl). Priming was performed on day 0 and booster on day 14. All mice were bled on days 0, 14 and 28 to analyse specific antibodies and sera were stored at -20°C.

Infection

A group of *Balb/c* mice ($n = 20$) was inoculated intraperitoneally according to Araj *et al*. (1977) with 200 μl of a suspension containing 2000 viable protoscoleces in PBS. Another group was inoculated with 200 μl of PBS as negative controls. All mice were bled on days 3, 7, 14 and 21 postinoculations and sera were stored at -20°C to analyse the antibody response.

Determination of specific antibodies

Anti-PSA or anti-$E4^+$ antibodies were measured by ELISA according to Míguez *et al*. (1996) in individual sera. Specific IgM was determined using goat anti-(mouse IgM) labelled with peroxidase (Sigma, St Louis, MO, USA). Specific IgG1, IgG2a, IgG2b and IgG3 titres were determined using appropriate dilutions of the corresponding goat anti-(mouse IgG) subclass antiserum (Nordik, Tilburg, The Netherlands) followed by rabbit anti(goat IgG) coupled to peroxidase (Sigma). Peroxidase activity was detected with 3-methyl 2-benzothyazolinone hydrazone hydrochloride and 3-dimethyl-aminobenzoic acid as chromophores (Sigma) (Ngo & Lenhoff 1980), and optical densities were measured at 600 nm (OD 600). A pool of positive sera was used as standard in all assays and OD 600 corresponding to each sample were converted to arbitrary units (AU) of antibody concentration, referred to this standard (Malvano *et al*. 1982).

SDS-PAGE and immunoblotting

PSA or $E4^+$ were resolved by SDS-PAGE under reducing conditions (Laemmli 1979) in 10% polyacrylamide gel and electrophoretically transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) (Towbin *et al*. 1979). Immunoblotting was performed as previously described (Severi *et al*. 1997) using peroxidase labelled goat anti-(mouse IgM) or goat anti-(mouse IgG) (Sigma) and developed with H_2O_2 and 3,3′-dimethylaminobenzidine (Sigma). Treatment of PSA transferred onto nitrocellulose membranes with sodium m-periodate (20 mM) was performed according to Hernández & Nieto (1994). After this treatment, the immunoblotting protocol described above was followed.

Lymphoproliferation assays

Spleen cells from individual mice were removed under sterile conditions. After washings, cells were resuspended in complete medium (RPMI 1640; 10% heat inactivated foetal calf serum; 0·05 mM 2-mercaptoethanol; 2 mM Hepes; 100 μg/ml streptomycin and 100 U/ml penicillin, all reagents from Sigma), in triplicates at a concentration of 2×10^5 cells per well and incubated with Con A (0·5 μg/well) (Sigma) or complete medium alone for 72 h, respectively, at 37°C in 5% CO_2. Before harvesting cells, 1 μCi of ^{3}H-thymidine (Amersham, Bucks, UK) was added to each well and

incubated for 16 h, followed by liquid scintillation counting. The proliferation index (PI) was calculated as: PI = mean c.p.m. of stimulated cells/mean c.p.m. of nonstimulated cells.

Determination of cytokines

Peritoneal cells were recovered from peritoneal lavages of infected or normal mice with 2 ml of cold RPMI. Spleen (5×10^6) or peritoneal cells (2×10^6) from individual mice were resuspended in complete medium dispensed in duplicates and incubated with 20 μg/ml of PSA, 20 μg/ml of $E4^+$ or complete medium alone in 24-well plates for 72 h at 37°C in 5% CO_2. After incubation, supernatants were recovered, centrifuged and stored at -70°C. The concentrations of IL-4, IL-10 and IFN-γ were determined by capture ELISA using commercial reagents (PharMingen, San Diego, CA, USA) following the manufacturer's instructions. Peroxidase activity was determined using *o*-phenylenediamine and optical densities were measured at 492 nm. Mouse recombinant IL-4, IL-10 and IFN-γ (Pharmingen) were used as standards.

Determination of the percentage of $CD3^+CD4^+$, $CD3^+CD8^+$ and sIg^+ cells

Splenocyte staining was performed according to standard protocols. Cells were incubated for 30 min at 4°C, with either phycoerythrin or fluorescein labelled anti-(mouse CD4), anti-(mouse CD8) or anti-(mouse CD3) monoclonal antibodies (Pharmingen), diluted in PBS containing sodium azide-0·5% bovine serum albumin. Unrelated monoclonal antibodies were used as negative controls. Fluorescein-labelled $F(ab')_2$-rabbit anti-(mouse immunoglobulins) (Dako, Glostrup, Denmark) was used to determine surface immunoglobulin positive cells (sIg^+). Cells were fixed in PBS containing 1% formaldehyde and fluorescence was analysed on a FACscan (Becton Dickinson, San Jose, CA, USA). The data were analysed with LYSYS II program with a scatter gate for lymphocytes and blast cells that excluded monocytes, polymorphs and dead cells.

Statistical analysis

Antibody titre data obtained from each group were compared by Student's *t*-test and cytokine concentrations were compared by Wilcoxon's test. Differences at $P<0{\cdot}05$ were considered statistically significant.

RESULTS

Immunogenicity of $E4^+$ in *Balb/c* mice

The specific antibody responses of *Balb/c* mice immunized with PSA (carbohydrate/protein content = 1/1) or $E4^+$ (carbohydrate/protein content = 4/1) were compared. As expected, PSA induced elevated titres of IgG1 whereas the carbohydrate rich fraction, $E4^+$ did not (Figure 1). Western blot analyses showed that most of the immunogenic epitopes of $E4^+$ components were sensitive to periodate oxidation suggesting that they were of glucidic nature, whereas both peptidic and glucidic epitopes from PSA components were immunogenic (Figure 2).

Suppression of lymphoproliferative response by $E4^+$

One of the biological activities found in many carbohydrate rich molecules is the suppression of the lymphoproliferative responses to mitogens (Giorgio *et al*. 1992, Persat *et al*. 1996). On the basis that $E4^+$ was enriched in carbohydrates and that immunosuppression was reported in *Balb/c* mice infected with *E. granulosus* (Allan *et al*. 1981), immunosuppressive activitiy in $E4^+$ components was analysed. As a preliminary approach, we determined the lymphoproliferative response to Con A of splenocytes from $E4^+$-immunized mice. The results showed that inoculation of $E4^+$ (but not PSA) suppressed the lymphoproliferative response to Con A both after priming and boosting (Figure 3). This suppression was not probably due to alterations within the percentages of B or T cells in spleens of $E4^+$-immunized mice according to results obtained by flow cytometry (Table 1).

Cytokine secretion induced by $E4^+$

The cytokine pattern induced by $E4^+$ was analysed in $E4^+$-immunized *Balb/c* mice in order to investigate whether $E4^+$ components could induce a polarized cytokine response. Interestingly, $E4^+$ induced IL-10 secretion by splenocytes from $E4^+$-immunized mice but failed to induce IL-4 (Figure 4). On the other hand, PSA stimulated IL-10 and IL-4 secretion. In addition, both antigens induced low levels of spontaneous IFN-γ secretion. These results suggest that $E4^+$ components may indirectly contribute to the type-2 cytokine pattern stimulated by whole homogenate protoscolex antigens (PSA), by downregulating type-1 cytokine production through the immunoregulatory activities of IL-10.

Immunogenicity of $E4^+$ in early infection

We have previously reported that $E4^+$ may contain T-independent antigens that stimulate IgM antibodies in CD4-depleted mice. Therefore, in this study, the immunogenicity of $E4^+$ in early infection was analysed by determining anti-$E4^+$ IgM titres. Results showed that $E4^+$ was immunogenic and that infected mice produced significantly

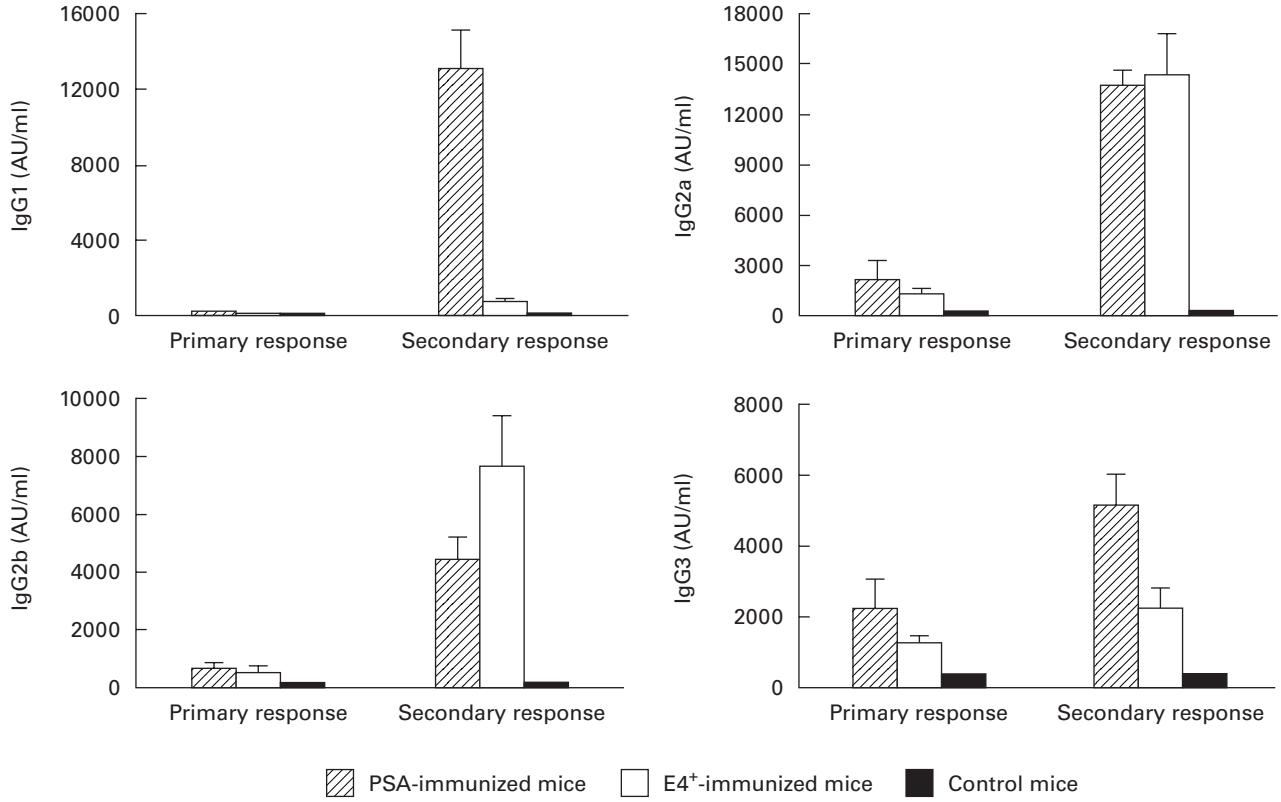

Figure 1 Isotypic profile of the antibody response stimulated by PSA or $E4^+$. Mice were inoculated on days 0 and 14 with PSA or $E4^+$ in PBS (50 μg of carbohydrates) intraperitoneally. Titres of anti-PSA IgG subclasses were determined in individual sera on day 14 (primary response) and 28 (secondary response) by ELISA and expressed as arbitrary units (AU/ml ± SEM) of concentrarion referred to a positive pool of sera used as standard.

elevated titres of anti-$E4^+$ IgM antibodies from day 3 post-infection (Figure 5). Western blot on $E4^+$ with sera from infected mice shows that a broad pattern of $E4^+$-components resulted immunogenic in the early stages of infection (Figure 6).

Cytokine secretion induced by $E4^+$ in early infection

To further investigate whether $E4^+$ may be involved in the polarization of a type-2 cytokine response in early infection with *E. granulosus*, we analysed the cytokines secreted *in vitro* by splenocytes from infected mice in response to $E4^+$. The results showed that $E4^+$ stimulated significantly elevated concentrations of IL-10 by splenocytes from infected mice on day 14 postinfection (Figure 7a) and did not induce secretion of IFN-γ or IL-4 (data not shown). One of the main *in vivo* source of IL-10 are B1 cells or macrophages and, since they constitute the major cellular population in peritoneal cavity, we further analysed whether $E4^+$ could stimulate IL-10 secretion by peritoneal cells. Results showed that *in vitro* stimulation of peritoneal cells from infected or PBS-inoculated mice with $E4^+$ also induced secretion of IL-10 at levels that were no different to those induced by PSA (Figure 7b). These results indicate a relevant role of $E4^+$ components in IL-10 secretion during the early stages of *E. granulosus* infection.

DISCUSSION

The immune response of *Balb/c* mice infected with *E. granulosus* is characterized by production of type-2 cytokines (Dematteis *et al*. 1999) and antibodies specific for carbohydrate epitopes (Ferragut & Nieto 1996, Míguez *et al*. 1996, Severi *et al*. 1997). The aim of this study was to investigate whether protoscolex glycoconjugates could participate in the induction of the type-2 cytokine response. For this purpose, the carbohydrate rich fraction $E4^+$, which we have previously described (Baz *et al*. 1999), was purified from PSA and used as a source of protoscolex glycoconjugates.

The first set of experiments were conducted to analyse the antibody and cellular responses stimulated by $E4^+$ in *Balb/c* mice. The results demonstrated that $E4^+$ was as immunogenic as PSA, except for IgG1 production, which was to be

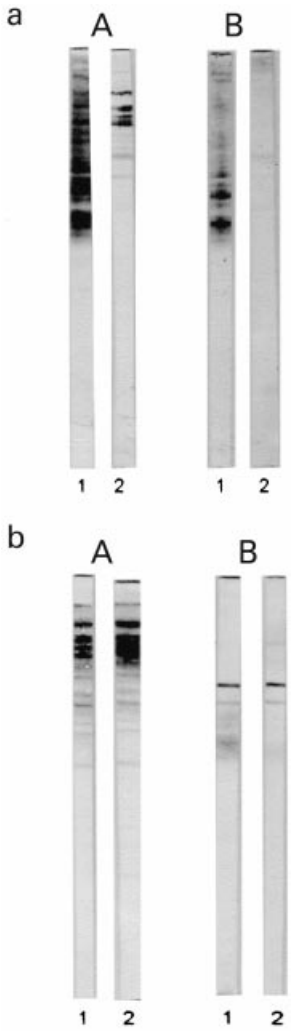

Figure 2 Recognition pattern of anti-PSA or anti-E4$^+$ IgM and IgG from inoculated mice. PSA was resolved by SDS-PAGE under reducing conditions and transferred onto nitrocellulose sheets. Sera from PSA (A) or E4$^+$-immunized (B) mice obtained from secondary response were incubated with m-sodium periodate treated (2) or nontreated PSA (1). Rabbit anti-(mouse IgM) (a) or anti-(mouse IgG) (b) immunoglobulins labelled with peroxidase were used as secondary antibody.

expected when taking into account that E4$^+$ is enriched in carbohydrates (Figure 1). The results from the antibody response also showed that E4$^+$ induced IgG2a but not IgG1, which may indicate that the response against E4$^+$ would be Th1-orientated rather than Th2-orientated. This was further investigated by analysing the cytokines secreted by splenocytes from E4$^+$-immunized mice. The cytokine pattern induced by E4$^+$ or PSA was consistent with the isotypic profile of the antibody response stimulated by each immunogen (Figure 4). Indeed, only PSA induced IL-4 driving the

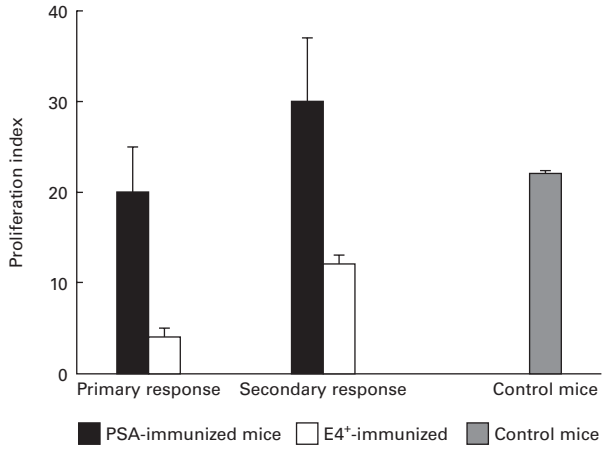

Figure 3 Proliferation index to Con A of splenocytes from mice inoculated with PSA or E4$^+$. Mice were inoculated on days 0 and 14 with PSA, E4$^+$ or PBS (50 μg of carbohydrates) intraperitoneally. Splenocytes were obtained on days 14 (primary response) and 28 (secondary response) and stimulated with Con A. Proliferation was determined by ^{3}H-thymidine incorporation and measured by liquid scintillation counting. Proliferation index (PI) was calculated as: PI = mean c.p.m. of stimulated cells/mean c.p.m. of nonstimulated cells.

switch to IgG1, whereas both PSA and E4$^+$ induced IL-10 secretion, which is involved in IgG3 switching (Shparago *et al*. 1996). Spontaneous secretion of IFN-γ by splenocytes from PSA or E4$^+$-immunized mice may account for IgG2a and/or IgG3 antibody production. Because the levels of IgG3 induced by PSA were higher than those induced by

Table 1 Percentages of B, CD4$^+$CD3$^+$ or CD8$^+$CD3$^+$ cells in spleens of PSA or E4$^+$-immunized mice

Immunogen*	sIg**	CD8$^+$CD3$^+$†	CD4$^+$CD3$^+$†
Primary response			
PSA	36 ± 1	13 ± 3	36 ± 3
E4$^+$	26 ± 4	15 ± 3	49 ± 10
Secondary response			
PSA	42 ± 3	16 ± 1	25 ± 1
E4$^+$	33 ± 14	24 ± 2	30 ± 4
PBS	38 ± 1	18 ± 7	30 ± 10

*Mice were inoculated on days 0 and 14 with PSA, E4$^+$ or PBS. **Data correspond to the percentage of surface immunoglobulin positive (sIg$^+$) cells in spleen of inoculated mice. †Data correspond to the percentage of CD4$^+$CD3$^+$ or CD8$^+$CD3$^+$ double positive cells in spleen of inoculated mice. Determinations were made on days 14 and 28 which correspond to primary or secondary responses, respectively. Data correspond to one out of two independent experiments and are expressed as the mean ± SD from five samples individually analysed.

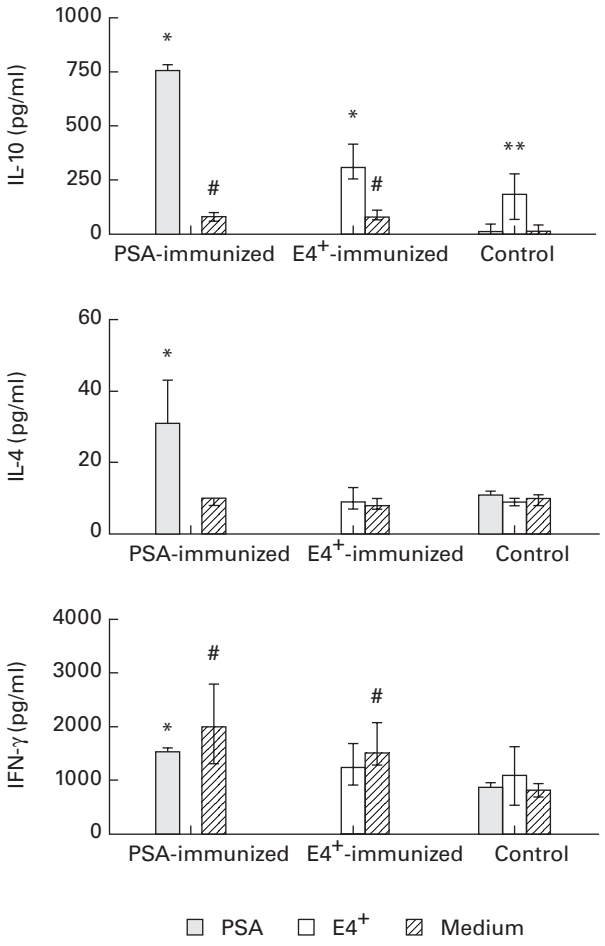

Figure 4 Cytokine pattern stimulated by PSA or $E4^+$ in *Balb/c* mice. Mice were inoculated on days 0 and 14 with PSA or $E4^+$ in PBS (50 μg of carbohydrates) intraperitoneally. Splenocytes from inoculated mice were cultured with PSA (dotted bars), $E4^+$ (white bars) or medium (striped bars) and cytokines were determined in individual culture supernatants by ELISA. Recombinant cytokines were used as standards. Control corresponds to cytokine concentrations obtained from mice inoculated with PBS. Bars represent the median of cytokine concentrations values and the vertical lines indicate the range of individual values from each group of mice. Data were compared by Wilcoxon's test and significant differences ($P < 0{\cdot}05$) are indicated as: (*) comparison of cytokine production by cells from inoculated mice cultured with antigen versus cells from control mice cultured with antigen; (**) comparison of cytokine production by cells from control mice cultured with antigen versus cells from control mice cultured without antigen; and (#) comparison of cytokine production by cells from inoculated mice cultured without antigen versus cells from control mice cultured without antigen.

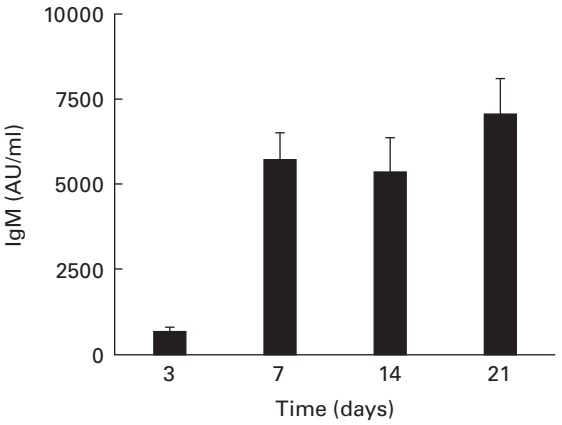

Figure 5 Immunogenicity of $E4^+$ in infected mice. Mice were infected with 2000 viable protoscoleces in PBS intraperitoneally on day 0. Titres of anti-$E4^+$ IgM antibodies were analysed by ELISA in individual sera on days 3, 7, 14 and 21. Titres are expressed as arbitrary units (AU/ml ± SEM) of concentrations referred to a pool of positive sera used as standard. Background levels obtained from sera of PBS-inoculated mice yielded 20 AU/ml.

$E4^+$, it is possible that PSA contains other immunogenic glycoconjugates in addition to those present in $E4^+$.

Interestingly, mice inoculated with $E4^+$ showed suppressed lymphoproliferative response to Con A. This biological activity may be related to immunosuppression and inhibition of lymphoproliferative responses to mitogens, as was previously reported in *E. granulosus* experimental infection (Allan *et al*. 1981, Riley *et al*. 1986) (Figure 3).

We can not conclude from our results that such immunosuppressive phenomena would be provoked by $E4^+$ components but it is an interesting hypothesis to explore further. Carbohydrate rich molecules involved in immunosuppression have been mainly described as glycolipids (Giorgio *et al*. 1992, Persat *et al*. 1996). Thus, the presence of such molecules in $E4^+$, and their putative association with immunosuppression, deserves further investigation. Components present in $E4^+$ may be toxic to lymphocytes reducing the Con A-driven proliferation of splenocytes from $E4^+$-immunized mice. However, such molecules were described in hydatid cyst fluid antigens but not in protoscoleces (Janssen *et al*. 1992). In addition, cells from inoculated mice showed viability percentages that were no different from those obtained with normal mice (data not shown). Suppression of proliferative responses was not due to alterations within the cellular composition of splenocytes, since percentages of T cells in spleens of $E4^+$-inoculated mice were not different to those obtained with control mice (Table 1). Impaired IL-2 secretion by splenocytes from $E4^+$-inoculated mice may not acccount for the reduced proliferation to Con A because splenocytes from $E4^+$-or PBS-immunized mice secreted similar concentrations of IL-2 (data not shown). Other mechanisms, e.g. that IL-2 was not biologically active, that $E4^+$-suppressive molecules could bind to IL-2 and block its activities (as occurs in the

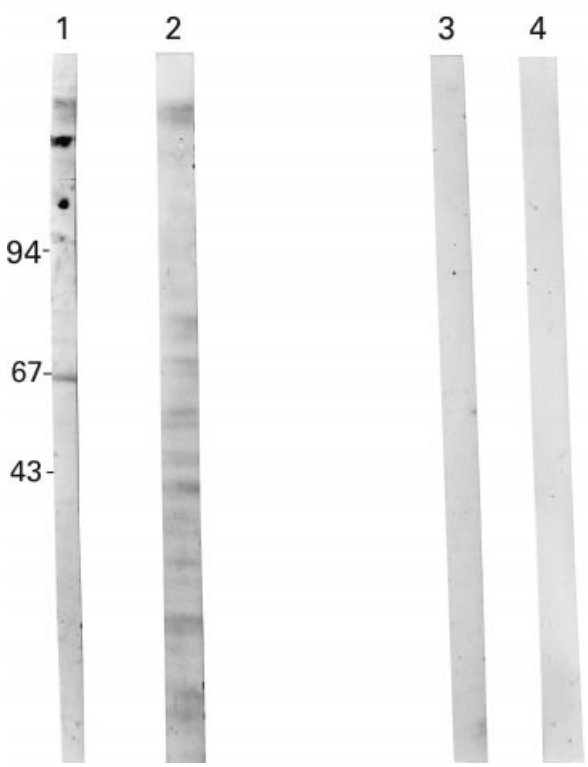

Figure 6 Recognition pattern of sera from infected mice on PSA or $E4^+$ by immunoblot. PSA (lanes 1 and 3) or $E4^+$ (lanes 2 and 4) were resolved by SDS-PAGE under reducing conditions and transferred onto nitrocellulose. Serum from an infected mouse obtained on day 21 postinfection was incubated with nitrocellulose sheet containing transferred PSA (lane 1) or $E4^+$ (lane 2). A serum from a noninfected mouse was used as negative control (lanes 3 and 4). Rabbit anti-(mouse IgM) immunoglobulins labelled with peroxidase were used as secondary antibody.

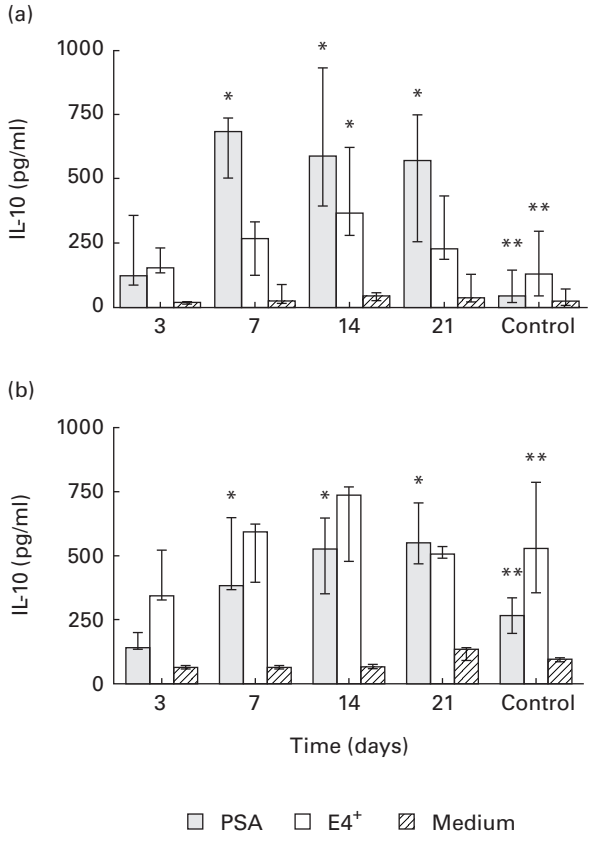

Figure 7 Secretion of IL-10 by spleen (a) or peritoneal cells (b) from infected mice. Mice were infected with 2000 viable protoscoleces in PBS intraperitoneally on day 0. Splenocytes or peritoneal cells from infected mice were cultured with $E4^+$ (white bars), PSA (dotted bars) or medium (striped bars) on days 3, 7, 14 or 21 postinfection. IL-10 concentration was determined by ELISA in individual supernatants. Control corresponds to IL-10 concentrations obtained from mice inoculated with PBS. Bars represent the median of cytokine concentrations values and vertical lines indicate the range of individual values from each group of mice. Data were compared by Wilcoxon's test and significant differences ($P < 0{\cdot}05$) are indicated as: (*) comparison of cytokine production by cells from infected mice cultured with antigen versus cells from control mice cultured with antigen; (**) comparison of cytokine production by cells from control mice cultured with antigen versus cells from control mice cultured without antigen.

case of gangliosides) or that splenocytes from $E4^+$-immunized mice expressed low levels of CD25, should be investigated. In addition, the possibility that $E4^+$ could stimulate T-$CD8^+$ suppresor cells should not be ruled out.

Suppresor activity could not be demonstrated in mice inoculated with PSA although PSA also contains $E4^+$ components. An explanation for this result could be that other components present in PSA (but not in $E4^+$) may compensate any suppresive activity or, alternatively, that those suppresor molecules would not be present in PSA at the appropriate concentration to induce immunosuppression.

In relation to the hypothesis that *E. granulosus* glycoconjugates may participate in the induction of a type-2 cytokine response, it is interesting to note that $E4^+$ induced the secretion of significantly elevated concentrations of IL-10 by normal or primed splenocytes, which may indicate that $E4^+$ could stimulate IL-10 secretion through a non-specific mechanism.

It is tempting to speculate that, in the early stages of *E. granulosus* experimental infection, which is performed by intraperitoneal inoculation of viable protoscoleces, $E4^+$ may stimulate peritoneal cells to secrete IL-10, a cytokine known to suppress the secretion of type-1 cytokines (O'Garra *et al*. 1996), and hence may contribute to initiate and sustain type-2 polarization. In this sense, the second set

of experiments was performed to provide further evidence on the role of $E4^+$ components in IL-10 production during infection. We analysed the immunogenicity of $E4^+$ and the cytokine profile induced by this fraction in early infection. The results showed that $E4^+$ was immunogenic during early infection (Figures 5 and 6) and that *in vitro* stimulation of splenocytes from infected or control mice with $E4^+$ induced secretion of significantly elevated levels of IL-10 compared to cells cultured with medium alone (Figure 7a). Because $E4^+$ also induced IL-10 secretion by cells from the peritoneal cavity (Figure 7b), where parasites were injected, its components may have an important role in the host–parasite interaction during the early stages of experimental infection, particularly in those events which may lead to polarization of the cytokine response.

Carbohydrate moieties, which were the most immunogenic epitopes in $E4^+$, might participate in the modulation of the cytokine response as well as proteic antigens. A role for carbohydrates in driving type-2 polarized responses, through IL-10 secretion by B1 cells, have been proposed as a general phenomenon occurring in infectious diseases (Velupillai & Harn 1994, Velupillai *et al*. 1997). It would be interesting to analyse whether this also occurs in *E. granulosus* infection and whether $E4^+$ components could have any role in such a phenomenon.

Apart from the cellular source of IL-10, the fact that $E4^+$ stimulates normal or primed peritoneal cells to secrete IL-10 may have a relevant role in *E. granulosus* infection. The epitope recognized by E492/G1 monoclonal antibody, used to obtain $E4^+$, is widely distributed in protoscoleces and is also present in *in vitro* protoscolex excretion/secretion (Baz *et al*. 1999). Therefore, we believe that $E4^+$ components may interact with host peritoneal cells at the very early stages of infection. Cells already present in the peritoneal cavity of mice would be induced to secrete IL-10 by live protoscolex inoculum depending on $E4^+$ *in vivo* availability, which may occur quite early in infection.

In summary, our results suggest that components present in the carbohydrate rich fraction $E4^+$ obtained from *E. granulosus* protoscoleces may be involved in immunosuppressive phenomena associated with hydatid infection. In addition, this fraction by stimulating IL-10 secretion, may contribute to the induction and sustaining of the type-2 cytokine response established in early experimental infection. Together with $E4^+$-driven IL-10 secretion, other components present in PSA that induce IL-4 secretion would also contribute to the type-2 response established in early *E. granulosus* infection. The demonstration of such mechanism and the identification of $E4^+$ and PSA molecules involved in IL-10 and IL-4 secretion, respectively, as well as their target cell populations, will be the objectives of future work.

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