International Immunology, Vol. 17, No. 6, pp. 721–728
doi:10.1093/intimm/dxh253

Co-infection with *Trypanosoma brucei brucei* prevents experimental autoimmune encephalomyelitis in DBA/1 mice through induction of suppressor APCs

Maja Wållberg and **Robert A. Harris**

Applied Immunology Unit, Centre for Molecular Medicine L8:04, Karolinska Institute, SE-17176 Stockholm, Sweden

Keywords: autoimmunity, immunomodulation, parasite

Abstract

The immune system has co-evolved with the infectious agents that challenge it, and in response pathogens have developed different mechanisms to subvert host immunity. A wealth of evidence suggests that infections are important components in the development of a functional immune system, and understanding the modulation of the host immune system by pathogens may offer new therapeutic strategies in a non-infectious setting. We investigated how infection with the protozoan parasite *Trypanosoma brucei brucei* (*Tbb*) modulates the autoimmune response to recombinant myelin oligodendrocyte glycoprotein (rMOG) in DBA/1 mice. Mice harbouring a *Tbb* infection did not develop experimental autoimmune encephalomyelitis (EAE) induced by immunization with rMOG in CFA, an animal model for the human autoimmune disease multiple sclerosis. Additionally, mice infected with the parasite at the time of immunization or 1 week later developed less severe EAE than uninfected controls. Protected mice displayed a markedly diminished rMOG-specific proliferation and IFNγ production in lymph node cells and had correspondingly low titres of serum anti-rMOG IgG. Antigen-presenting cells (APCs) from spleens of *Tbb*-infected mice presented rMOG less efficiently to rMOG-specific T cells *in vitro* than did splenic APCs from uninfected mice and could also inhibit antigen-specific proliferation in control *in vitro* cultures. This suppressive effect is at least in part due to increased release of IL-10. Transfer of splenic APCs from *Tbb*-infected mice into mice immunized with rMOG-CFA 7 days previously abrogated disease significantly. These findings indicate that infections can prevent autoimmunity and that APCs might be used as immunomodulants.

Introduction

The immune system is constantly exposed to infectious agents, shaping the repertoire of immunocompetent cells as well as influencing the setting in which they act. Autoimmune disorders such as multiple sclerosis (MS), rheumatoid arthritis and insulin-dependent diabetes mellitus (IDDM) develop as a result of inappropriate, self-directed immune activity. It has been hypothesized that one potential reason for the increase in frequency of such autoimmune diseases as well as of allergies in the Western World is the decreased incidence of chronic infection that results from the improvement of living standards that has taken place during the last few centuries. This hypothesis is commonly referred to as the 'hygiene hypothesis,' and has received support in both epidemiological and experimental settings (1–3).

In the human disease MS, infiltrating immune cells cause demyelination and neuronal damage in the central nervous system. The inflammation is believed to be of autoimmune origin, even though infectious agents have been suggested to have a role in initiation of disease (4–6). A predominating concept when discussing autoimmune responses has been the Type 1–Type 2 (T1–T2) cytokine dichotomy. Many autoimmune disorders are considered to be T1 dominated, with IFNγ and tumour necrosis factor playing central roles in disease progression, while allergic responses are considered

Correspondence to: M. Wållberg; E-mail: maja.wallberg@cmm.ki.se

Received 22 November 2004, *accepted* 7 March 2005

Transmitting editor: A. Cooke

Advance Access publication 17 May 2005

to be skewed toward a T2 profile (7, 8). T1 immune responses, and especially presence of IFNγ, have been identified as being important in MS progression (9), although caution is warranted when applying the T1–T2 dichotomy in the human situation (10). In concordance with this cytokine balance theory, infectious agents inducing T2 cytokines such as IL-4 and IL-10 should have a dampening effect on autoimmunity, but an accelerating effect on allergic responses. The efficacy of cytokine modulation in abrogating autoimmunity has indeed been previously demonstrated (6, 7). However, the fact that there have been parallel increases in the incidence of allergy and autoimmunity, both predominantly in urban areas (11), seems to question this prediction and rather indicates that reduced exposure to both T1- and T2-inducing pathogens may lead to disordered immunoregulation, leading to development of autoimmunity and allergy.

Microbial infection can impact on the course of autoimmune disease, both in disease-inducing and disease-protecting capacities. For example, epidemiological evidence indicates that rheumatic fever may follow streptococcal infection (12) and that *Trypanosoma cruzi* infection is the instigator of the chronic heart condition known as Chagas' disease (13). Conversely, accumulating evidence suggests that infection may abrogate development of autoimmune pathology. In animal models, it has been demonstrated that infection with the helminth *Schistosoma mansoni* can inhibit development of IDDM in the non-obese diabetic (NOD) mouse (14) and experimental autoimmune encephalomyelitis (EAE) in mice (15). Injection of *S. mansoni* eggs also abrogates IDDM in NOD mice (16) and EAE (17), and in the NOD mouse even injection of soluble extracts from the eggs can lead to prevention of disease (16). The eggs of *S. mansoni* are known to induce a T2-biased immune response in the host (18), and as both IDDM in the NOD mouse and EAE are believed to be T1-mediated, it is an attractive strategy to modulate the destructive immune responses with a pathogen inducing the diametrically opposite cytokine profile. The protective effects in the studies cited are indeed believed to be caused by a T2 shift in the host's immune responses (16, 17), but it is also argued that modulation of antigen-presenting cell (APC) activation may be important (15, 16).

The protective effects of infections with pathogens associated with T1, rather than T2 immunity are harder to explain according to this theory. In studies of amelioration of EAE following infection with T1-inducing *Mycobacterium bovis* (19), the effect is reported to be caused by changed trafficking of autoreactive T cells. Similar studies of inhibition of IDDM in NOD mice (20, 21) indicate induction of anergy or deletion of the autoreactive T cells as the mechanism underlying the protective effect.

Trypanosoma brucei brucei (*Tbb*) is an extracellular parasite infecting cattle in sub-Saharan Africa that also induces a prominent T1 response. Infection eventually leads to potent immunosuppression of the host, a phenomenon attributed to the induction of suppressor macrophages (22–24). In a previous study we reported that concurrent infection with *Tbb* can modulate the development of experimental collagen-induced arthritis (CIA), although the underlying mechanism for this parasite-induced suppression of autoimmunity was not discerned (25). In the current study we have similarly explored the immunomodulatory effect of concurrent infection with *Tbb* on development of recombinant myelin oligodendrocyte glycoprotein (rMOG)-induced EAE in DBA/1 mice (26). We found that infection with this IFNγ-inducing parasite abrogated rMOG-EAE and inhibited both humoral and cellular immune responses to rMOG. We further demonstrate that this effect was due to the action of suppressor APCs induced by the infectious agent and the associated production of IL-10.

Methods

Animals

Female DBA/1 mice were purchased from Harlan (Netherlands) or bred in the animal facility at MTC, Karolinska Institute, and used at 8–12 weeks of age. They were kept in the animal facility at the Karolinska Hospital, where the conditions were specific pathogen-free, and had free access to chow and water. All experiments were performed in accordance with the approval of ethical committee Stockholm North.

Reagents

Recombinant protein corresponding to the N-terminal sequence of rat MOG (amino acids 1–125), hereafter referred to as rMOG, was expressed in *Escherichia coli* and purified by metal chelate chromatography as described (27). Purified protein was dialyzed into sodium acetate (10 mM, pH 3.0) and stored at −20°C. Incomplete Freund's adjuvant and heat-killed *Mycobacterium tuberculosis* (MT) H37Ra were purchased from Difco Laboratory (Detroit, MI, USA).

Parasites

Stock stabilates of *Tbb* AnTat1.1E were diluted in sterile PBS to 10^6 parasites ml^{-1} and 50 μl of this solution was injected intraperitoneally (i.p.) into each mouse, giving a dosage of 5×10^4 parasites per mouse.

Induction and clinical evaluation of EAE

Mice were anaesthetized with isoflurane and injected intradermally in the tail base with 50 μg rMOG emulsified in Freund's adjuvant containing 200 μg MT per dose. Starting from day 10 post-immunization, the mice were weighed and assessed for signs of disease daily as follows: 0 = no detectable signs of disease, 1 = tail paralysis, 2 = hind limb paraparesis, 3 = hind limb paralysis, 4 = complete paralysis of hind and fore limbs and 5 = dead.

Proliferation assays

Inguinal lymph nodes were harvested from control mice or *Tbb*-infected mice immunized with rMOG-CFA 5 days previously. Single-cell suspensions were prepared from lymph nodes and re-suspended in DMEM medium supplemented with 10% FCS, 1 mM sodium pyruvate, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (all reagents from Life Technologies, Paisley, Scotland). Cells were cultured in triplicate in U-bottomed 96-well plates (Costar, Cambridge, MA, USA), 2×10^5 cells per well, in the presence of rMOG (40 μg ml^{-1}) at 37°C in a humidified atmosphere containing

5% CO_2 for 72 h, with [^{3}H]thymidine ([^{3}H]TdR; Amersham International, UK; 1 μCi per well) being added for the last 18 h of culture. Cells were harvested using a Tomtec cell harvester (Wallac Oy, Turku, Finland) and incorporated radioactivity was detected using a β-liquid scintillation counter, 1450 Microbeta Plus (Wallac Oy).

Antigen presentation studies

For antigen presentation studies, APCs were purified using anti-MHC II beads (MACS, Miltenyi) from spleens of control mice and mice infected with *Tbb* 2 weeks previously, which were subsequently seeded into 24-well plates (Nunc, Denmark) at a density of 2×10^6 cells per well and cultured in DMEM with the usual additives and rMOG (40 μg ml^{-1}) for 24 h. Cells were then washed, counted, irradiated (25 Gy) and seeded in 96-well plates at a density of 20×10^3 cells per well.

T cells were purified from draining lymph nodes of mice immunized with rMOG-CFA 5 days previously and added to the APCs at a density of 10×10^4 per well. The T cells were separated using anti-Thy1 microbeads (MACS) according to instructions from the manufacturer. Briefly, cells were incubated for 15 min with 20 μl of bead preparation for every 10^7 cells, washed and applied to the separation column. The purity of the obtained cell populations was determined through flow cytometric analysis using FITC-conjugated anti-CD3 (BD, San José, CA, USA) and biotinylated anti-I-Aq (BD) followed by incubation with PE-conjugated streptavidin (Serotec, Raleigh, NC, USA).

After addition of T cells, the cultures were incubated for 72 h, with [^{3}H]TdR (Amersham International) (1 μCi per well) being added for the last 18 h of culture. Cells were harvested using a Tomtec cell harvester (Wallac Oy) and incorporated radioactivity was detected using a β-liquid scintillation counter, 1450 Microbeta Plus (Wallac Oy).

Suppression studies

For suppression studies, spleen cells from control mice and mice infected with *Tbb* 2 weeks previously were purified and enriched for MHC II using microbeads (MACS, Miltenyi). After separation, cells were washed, counted and seeded into 24-well plates at a density of 2×10^6 cells per well. Co-culture of control APCs and *Tbb*-APCs was set up at a 1 : 1 ratio with a final density of 2×10^6 cells per well. The cells were co-incubated for 8 h, pulsed with rMOG (40 μg ml^{-1}) overnight, and then washed, counted and irradiated (25 Gy). A total of 40×10^3 cells were added per well to ensure that the equivalent numbers of control APCs (20×10^3) as in the control cultures would be added. T cells were purified and added as described above. Proliferation was determined through incorporation of [^{3}H]TdR as described above.

For studies of the suppressive effects of supernatants, spleen cells from *Tbb*-infected mice were cultured in 24-well plates (2×10^6 cells per well) for 18 h, then supernatants were harvested, sieved through a 0.2-μm cell strainer to remove contaminating cells and added to control cultures. When indicated, supernatants were pre-incubated with either neutralizing anti-IL-10 antibody or isotype control (both BD) at a concentration of 25 μg ml^{-1} for 1 h previous to addition to cultures. The cells were then incubated overnight, after which they were pulsed with rMOG for 24 h, harvested, washed, counted, irradiated (25 Gy) and re-seeded in 96-well plates at a density of 20×10^3 cells per well. T cells were purified and added as described above. Proliferation was determined through incorporation of [^{3}H]TdR as described above.

Transfer of splenic APCs

APCs were purified using anti-MHC II beads (MACS) from spleens of mice infected with *Tbb* 2 weeks previously. For controls, APCs were purified from mice immunized with ovalbumin peptide (OVA) at the same time point. A total number of 2×10^6 cells per mouse were injected i.p. into mice that had been immunized with rMOG-CFA 7 days previously.

TaqMan PCR

Inguinal lymph nodes were harvested from control mice or *Tbb*-infected mice immunized with rMOG-CFA 5 days previously. Cells were re-suspended in DMEM supplemented with 10% FCS and antibiotics with or without rMOG (40 μg ml^{-1}). After 24 h of culture at 37°C the cells were harvested, washed and total RNA was extracted according to instructions in the RNeasy Mini Kit (Qiagen) with additional incubation with DNase (Qiagen) to avoid contamination of genomic DNA. Reverse transcription was performed with 10 μl RNA, random hexamer primer (0.1 μg ml^{-1}, GIBCO BRL) and SuperScript Reverse Transcriptase (200 U, GIBCO BRL). The mouse IFNγ primers and probe were designed using Primer Express software (Applied Biosystems) avoiding contaminating genomic DNA amplification by positioning one of the primers over an exon/intron boundary, and then ordered from SGS DNA (Köping, Sweden). IFNγ: 5′-primer GCATAGATGTGGAAGAAAAGAGTCTCT, 3′-primer CTGGCTCTGCAGGATTTTCAT, probe [6-carboxy-fluorescein (FAM)]-TCACCATCCTTTTGCCAGTTCCTCCAG. The probe was labelled with FAM at the 5′ end as reporter dye and 6-carboxy-tetramethyl-rhodamine(TAMRA) at the 3′ end as quencher dye except for the 18 S rRNA probe, which was labelled with VIC as reporter dye and TAMRA as quencher dye. Amplification was performed using *Taq*Man methodology with a two-step PCR protocol (pre-incubation 10 min with 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 1 min) on the ABI PRISM 7700 Sequence Detector (Applied Biosystems). The ribosome 18 S unit cDNA was used as a stable endogenous control. The relative quantification was performed using the standard curve method. Standard curves were constructed using three 100-fold dilutions of standard sample (cDNA Con A stimulated mouse lymphocytes) and corresponding cycle of threshold value. The samples were run in triplicates. After computing relative amounts of cytokine cDNA and endogenous control for one sample, the final amount was represented as ratio between the relative amount of cytokine cDNA and the relative amount of endogenous control cDNA, 18 S rRNA.

Serum ELISA

Serum samples were collected day 16 post-immunization or earlier if the mice had to be sacrificed due to severe paralysis (day 12–16 post-immunization). Serum ELISA was conducted as described (26) using biotinylated goat anti-mouse IgG (Dako, Denmark) for total IgG. Plates were coated with rMOG (2.5 μg ml^{-1}), developed with ABC and

3,3′,5,5′-tetramethylbenzidine (Sigma), stopped with 1 M HCl and read at 450 nm using a microplate reader (Labsystem).

Cytokine ELISA

Analysis of supernatant IFNγ and IL-10 was performed using the duokit sandwich ELISA systems from R&D (Minneapolis, MN, USA) according to the protocols provided by the manufacturer.

Results

Concurrent infection with Tbb abolishes development of EAE in DBA/1 mice

We first assessed whether microbial activation of the immune system would impact on development of MOG-EAE. Mice harbouring a *Tbb* infection did not develop EAE upon immunization with rMOG in CFA, and mice that were co-infected with the parasites at the time of immunization or a week later developed less severe EAE than uninfected controls (Fig. 1A). Administration of dead parasites did not have any protective effect unless mixed into the inoculum itself (Fig. 1B). This effect is interesting in its own right and has been observed with other non-parasite antigens such as OVA (28) and keyhole limpet haemocyanin (29). The fact that this effect can be observed with non-parasite-derived antigens makes it very unlikely that it has importance for the suppression evident with *Tbb* co-infection.

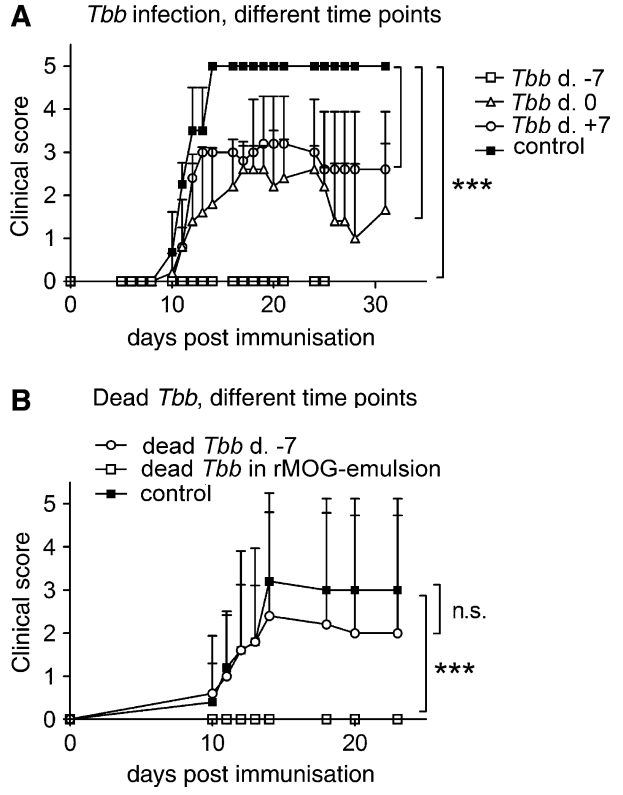

Fig. 1. Infection with *Tbb* abrogates development of EAE, but injection of dead parasites does not. An i.p. injection of 5×10^4 live parasites 7 days before immunization with rMOG-CFA completely inhibits development of signs of EAE (A). Infection on the same day as or 7 days after immunization led to milder EAE than in controls (A). Injection of the same number of dead parasites could not abrogate disease unless added into the rMOG-CFA inoculum (B). There were five mice in each group and the data are representative of three independent experiments with consistent results. Statistical analysis was performed using the Mann–Whitney test, $***P < 0.005$. Error bars represent standard deviation.

Infection with Tbb prevented mice from developing both cellular and humoral responses to rMOG

In vitro assays were used to establish whether the immunosuppressive effect was evident in altered cellular responses. EAE-protected mice displayed a markedly diminished antigen-specific proliferation to rMOG in inguinal lymph node cells harvested 7 days post-immunization with rMOG-CFA (Fig. 2A). Mice infected at day −7 also displayed lower levels of IFNγ-transcripts in response to rMOG (Fig. 2B) and had lower titres of serum anti-rMOG IgG than did controls, with the lowest levels of specific antibodies being recorded in mice infected 7 days prior to immunization with rMOG in CFA (Fig. 2C).

Presentation of rMOG is compromised in APCs from Tbb-infected mice

Earlier studies have demonstrated that immunosuppression in *Tbb*-infected hosts is due, at least in part, to development of suppressor macrophages (22, 30). The ability of macrophages

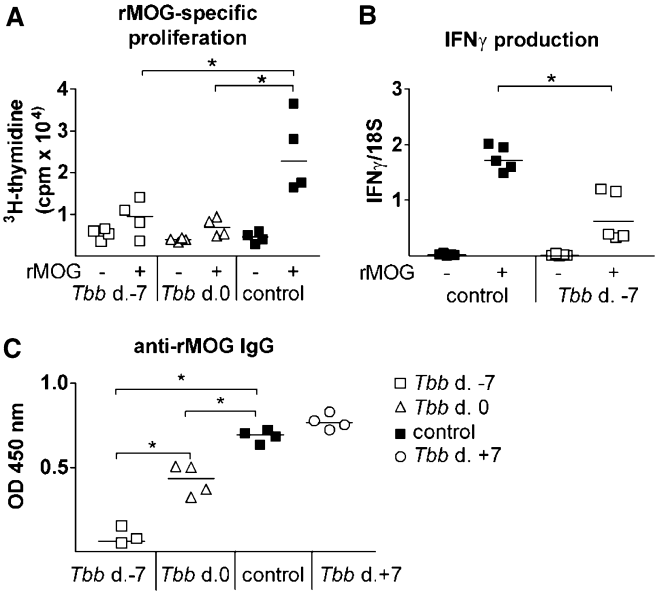

Fig. 2. Infection with *Tbb* suppresses both humoral and cellular responses to rMOG. Recall proliferation to rMOG was compromised in infected animals (A), both when they were infected 7 days prior to and at the same time as immunization. Levels of IFNγ message in response to rMOG were lower in co-infected mice (B). Results are presented as ratio of IFNγ message compared with endogenous control ribosomal RNA unit 18S. Mice infected with *Tbb* either 7 days before or at the same time as immunization with rMOG-CFA had lower anti-rMOG IgG titres than controls (C). Anti-rMOG IgG levels were not affected in mice infected 7 days after immunization (C). Results are presented as optical density detected at 450 nm. Statistical analysis was performed using the Mann–Whitney test, $*P < 0.05$. In all experiments there were 4–5 mice per group and data are representative of at least two independent experiments.

from *Tbb*-infected mice to present rMOG to rMOG-specific T cells was thus investigated. Purified APCs from *Tbb*-infected mice and controls were pulsed with rMOG overnight, then washed, irradiated and seeded into 96-well plates. Purified T cells from the draining lymph nodes of mice immunized with rMOG-CFA 5 days previously were added to the cultures in a recall proliferation assay. APCs from infected mice did not induce either rMOG-specific proliferation (Fig. 3A) or IFNγ production (Fig. 3B) as efficiently as those from non-infected animals. IFNγ production was not detectable in non-stimulated cultures from either group (data not included).

Co-incubation with APCs from infected mice diminishes the capacity of control APCs to induce rMOG-specific responses in lymphocytes

To further elucidate the nature of the suppressor APC phenotype, we investigated the ability of *Tbb*-induced suppressor APCs to inhibit antigen-specific cellular recall response *in vitro*. Pre-incubation with APCs from *Tbb*-infected mice in the cell culture inhibited control APCs from inducing rMOG-specific proliferation (Fig. 4A) and IFNγ production (Fig. 4B) when added in culture with purified rMOG-specific T cells, even though double the number of (40×10^3 instead of 20×10^3) APCs were used to eliminate the risk of decreased proliferation and IFNγ production merely due to a dilution effect. IFNγ production was not detectable in non-stimulated cultures from either group (data not included).

Secretion of IL-10 is an important component in Tbb-APC-mediated suppression of rMOG-specific responses

As reported earlier (31), *Tbb* infection can lead to immunosuppression through secretion of IL-10. Spleen cells from *Tbb*-infected mice do indeed produce more IL-10 than controls, as determined in cell culture supernatants by ELISA (Fig. 5A). To determine if APCs from infected mice suppress immune responses to rMOG through secretion of soluble factors, we transferred supernatants from cultures of APCs from infected mice into control cultures. Transfer of supernatants did indeed inhibit the control APCs from activating rMOG-reactive T cells as determined through rMOG-specific proliferation (Fig. 5B) and IFNγ production (Fig. 5C). Pre-incubation of the supernatant with anti-IL-10 antibody abolished the suppressive effect, while pre-incubation with an isotype control antibody did not affect suppression (Fig. 5B and C). IFNγ production was not detectable in non-stimulated cultures from either group (data not included). There was a tendency, although not statistically significant, that activation in cultures where anti-IL-10 antibody had been added was not totally restored. Thus, it cannot be excluded that other factors may contribute to the suppressive effects.

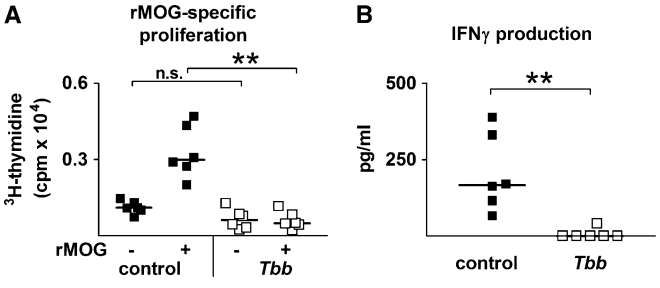

Fig. 3. Splenic APCs from *Tbb*-infected mice are compromised in their capacity to present rMOG. Purified APCs from control mice or from mice infected with *Tbb* 14 days previously were pulsed with rMOG for 24 h. Cells were then washed, irradiated and seeded in 96-well plates, after which purified T cells from draining lymph nodes of mice immunized with rMOG-CFA 5 days previously were added. APCs from the infected mice were less efficient at activating rMOG-specific T cells than control APCs both regarding proliferation (A) and IFNγ production (B). Positive antibody-mediated selection of MHC II-positive cells from *Tbb*-infected mice yielded populations comprising between 70 and 94% MHC II$^+$ cells as determined by FACS analysis, and similar purification from control spleens yielded populations consisting of 81–95% MHC II$^+$ cells. Selection for Thy1$^+$ cells yielded populations of 67–97% CD3-positive cells. Statistical analysis was performed using the Mann–Whitney test, $^*P < 0.05$, $^{**}P < 0.01$. Each group contained six cultures, and results are representative of three independent experiments.

Transfer of APCs from infected mice abolishes development of EAE

The capacity of *Tbb*-induced suppressor APCs to modify development of autoimmunity was next investigated *in vivo* in adoptive transfer experiments. Transfer of APCs from *Tbb*-infected mice into mice immunized with rMOG-CFA 7 days previously inhibited disease development significantly, while transfer of APCs from mice previously injected with OVA did not protect the mice (Fig. 6). No parasites were detected among the cells separated for transfer, but it cannot be excluded that a small number may have been transferred. However, as the mice were continually checked for parasitaemia and tested negative, we consider the effect to be due to the transferred cells rather than to any marginal parasite contamination. These results indicate that parasite-induced suppressor APCs have the ability to abrogate an ongoing autoimmune response.

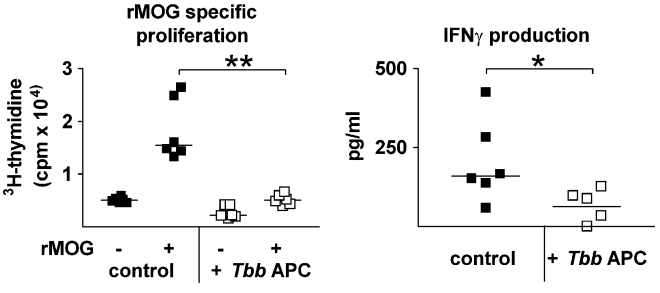

Fig. 4. APCs from infected mice suppress function in control APCs when added into the culture. When purified control APCs were co-incubated with APCs from infected mice at a ratio of 1 : 1 prior to and during pulsing with rMOG, their capacity to induce rMOG-specific proliferation (A) and IFNγ production (B) in rMOG-specific T cells was reduced. APC numbers added to the cultures were adjusted so that 20×10^3 control APCs were added. MHC II$^+$ cells were 91–95% of the purified populations. Selection for Thy1$^+$ cells yielded populations of 95–97% CD3-positive cells. Statistical analysis was performed using the Mann–Whitney test, $^*P < 0.05$, $^{**}P < 0.01$. Each group contains six cultures, and results are representative of three independent experiments.

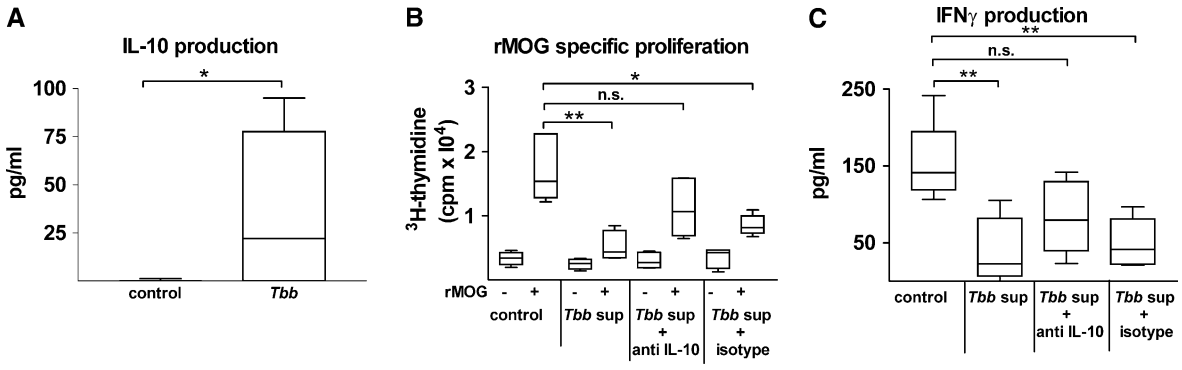

Fig. 5. APCs from infected mice suppress control APC function through secretion of IL-10. Purified splenic APCs from infected mice secrete higher levels of IL-10 than control APCs (A). Addition of supernatants from *Tbb*-APC cultures suppress APC function of control APCs as determined through their capacity to induce rMOG-specific proliferation (B) and IFNγ production (C) in rMOG-specific T cells. The suppressive effect was reversed when supernatants were pre-incubated with neutralising anti-IL-10 antibody, but not when these were pre-incubated with an isotype control antibody (B, C). MHC II$^+$ cells were 91–95% of the purified populations. Selection for Thy1$^+$ cells yielded populations of 95–97% CD3-positive cells. Boxes extend from the 25th to the 75th percentiles, the middle line represents the median and whiskers show the range of the data. Statistical analysis was performed using the Kruskal–Wallis test, $*P < 0.05$, $**P < 0.01$. Each group contains six cultures, and results are representative of two independent experiments.

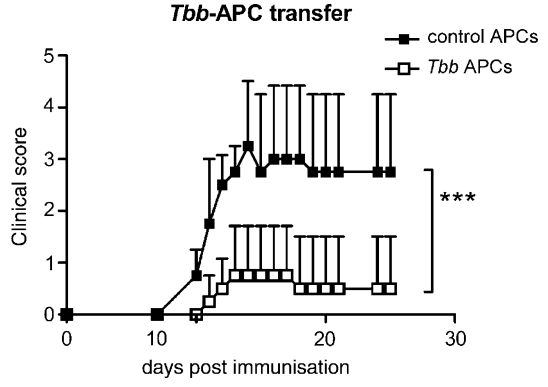

Fig. 6. Transfer of splenic APCs from *Tbb*-infected mice inhibits EAE. Mice were immunized with rMOG emulsified in CFA. Seven days post-immunization, they received 2×10^6 splenic APCs derived from either the spleens of mice that had been infected with *Tbb* (purity 91% MHC II$^+$ cells) or immunized with OVA emulsified in CFA (purity 95% MHC II$^+$ cells) 2 weeks previously. Mice receiving the *Tbb*-APCs were protected from developing EAE compared with the controls receiving OVA-APCs. Statistical analysis was performed using the Mann–Whitney test, $***P < 0.005$. The groups contained at least four mice, and the data are representative of three independent experiments. Error bars represent standard deviation.

Discussion

In this study, we have investigated the immunomodulatory effect of concurrent infection with *Tbb* on the development of rMOG-EAE. We report that infection with this pro-inflammatory cytokine-inducing parasite abrogated rMOG-EAE and inhibited both humoral and cellular rMOG-specific immune responses. We further demonstrated that this effect was due to the action of suppressor APCs induced by the infectious agent. These suppressor APCs were not only less efficient at activating rMOG-specific T cells, but could also suppress APC function in control APCs. This effect was, at least in part, due to soluble factors and especially IL-10. Finally, we demonstrated that the suppression of rMOG-EAE development could be achieved through adoptive transfer of *Tbb*-induced suppressor APCs.

In earlier studies, the capacity of *Trypanosoma* spp. to decrease responsiveness to vaccination (32, 33), antigen-specific responses in general (22, 31) as well as autoreactive responses in CIA (25), has been described. This immunosuppression has been attributed to disparate mechanisms—suppressor macrophages, which either act through production of NO, thus affecting the T cells they come into contact with (23, 24, 34), through secretion of IL-10 (31) or an inability to effectively co-express MHC II and peptides on their surface (31). Other studies have identified decreased secretion of NO due to altered arginine catabolism as a mechanism for suppression, generating a phenotype called 'alternatively activated macrophages' that characterizes late stages of infection with a genetically modified variant of *Tbb* (35). In general, NO has been associated with immunosuppression during the early stages of infection, with other less well-characterized mechanisms being more important in later stages (36, 37). In our study, we could attribute the immunosuppressive effect to parasite-modulated APCs, detect elevated levels of IL-10 in cultures containing APCs from infected mice and could also conclude that the elevated levels of IL-10 were important for induction of the recorded immunosuppression.

It has previously been determined that antigen uptake is not compromised in APCs from infected mice (31), leading to the conclusion that parasite antigens competing with the MOG-peptides for binding to the MHC II could be partially responsible for the lower recall proliferation in cultures in which APCs from infected mice are used.

However, the impaired capacity to present rMOG to rMOG-specific T cells cannot account for the suppressive effect of the APCs from infected mice when added to other recall cultures. This effect was determined to be caused by soluble factors and primarily IL-10. This cytokine was first described as a factor inhibiting T1 responses (38) and has since been identified as an important immunoregulatory cytokine affecting

activation and effector function of T cells and APCs (39). In experiments *in vivo*, IL-10-deficient mice develop more severe EAE while transgenic mice over-expressing IL-10 under the CD2 promoter are protected (40), and administration of IL-10 during the initial phases of the immune response and disease mitigates EAE development (41, 42). Transgenic expression of IL-10 under the MHC II promoter protects mice from developing EAE upon immunization with spinal cord homogenate (43). As over-expression in this case is specific for APCs, it indicates how important the levels of IL-10 in the APCs are for disease progression.

The concept of modulating immune responses to self-antigens by infecting the host with various pathogens has been investigated in recent years. Observations of impaired vaccination efficiency in populations afflicted by *Nippostrongylus* infections, and of how vaccination efficiency was restored after anti-parasite treatment (44) elegantly demonstrate this modulating effect. Another groundbreaking finding is the immunomodulatory efficacy of administration of *Trichuris suis* eggs to patients suffering from IBD, leading to amelioration of their symptoms (45). The focus of such investigations has mainly been on possibilities of skewing the T1–T2 responses in a favourable direction, based on the idea that many autoimmune disorders are associated with T1-biased responses. As this concept has attracted more and more criticism (8), and investigators have questioned whether the T1–T2 dichotomy really is a fruitful concept when trying to understand human MS pathology (10), the concept of how infections may influence autoreactivity has also been modified (2). Although there is convincing evidence for a protective effect of conventional T2 cytokine skewing using parasites in mouse EAE (17) and NOD mouse IDDM (16), there are additional findings playing down the importance for T2 cytokines, favouring other means of modulation. In studies of NOD mouse IDDM for instance, Martins and co-workers reported that administration of T1 inducer *Mycobacterium avium* could abrogate development of disease through induction of T1 immunity (20, 21), and Sewell and co-workers discovered that infection with *M. bovis* (19) can abrogate development of EAE in mice through influencing localization of pathogenic T cells in the host. The same authors also found that administration of heat-killed MT, the classical component used in CFA to induce T1 immunity, could mitigate EAE development (46).

An interesting immunoregulatory perspective is that presented by Barthlott and co-workers (47), suggesting that regulation of autoreactive T cells can be achieved by any activated T cells without the need for expression of mediators classically associated with regulatory T cell function. The notion that regulation could be in large part a question of homeostasis within the immune system and that a first step in preventing autoreactivity would be to have enough non-pathogenic T cells dividing to compete with the autoreactive ones for common resources is very attractive and much in concordance with the hygiene hypothesis.

In recent years, epidemiological investigations as well as applied studies in animal models and patients have suggested that infections may have a modulating effect on the development and perpetuation of autoimmune responses. Once elucidation of the immunological mechanisms underlying modulation are characterized and protocols established for their duplication *in vitro*, then new therapies can be established that mimic this fascinating natural immunoregulation.

Acknowledgements

This work was supported by the Swedish Medical Research Council, the King Gustav V 80 year fund, the Swedish Association of Neurologically Disabled and Karolinska Institutet fonder.

Abbreviations

APC	antigen-presenting cell
CIA	collagen-induced arthritis
EAE	experimental autoimmune encephalomyelitis
FAM	6-carboxy-fluorescein
[^{3}H]TdR	[^{3}H]thymidine
IDDM	insulin-dependent diabetes mellitus
i.p.	intra-peritoneal
MS	multiple sclerosis
MT	*Mycobacterium tuberculosis*
NOD	non-obese diabetic
OVA	ovalbumin peptide
rMOG	recombinant myelin oligodendrocyte glycoprotein
TAMRA	6-carboxy-tetramethyl-rhodamine
Tbb	*Trypanosoma brucei brucei*

References

1 Sewell, D. L., Reinke, E. K., Hogan, L. H., Sandor, M. and Fabry, Z. 2002. Immunoregulation of CNS autoimmunity by helminth and mycobacterial infections. *Immunol. Lett.* 82:101.
2 Rook, G. A., Ristori, G., Salvetti, M., Giovannoni, G., Thompson, E. J. and Stanford, J. L. 2000. Bacterial vaccines for the treatment of multiple sclerosis and other autoimmune disorders. *Immunol. Today* 21:503.
3 Thomas, T. D. C., Zaccone, P., Dunne, D. W. and Cooke, A. 2004. The impact of infection on the incidence of autoimmune disease. *Curr. Top. Med. Chem.* 4:521.
4 Wucherpfennig, K. W. 2001. Mechanisms for the induction of autoimmunity by infectious agents. *J. Clin. Investig.* 108:1097.
5 Wucherpfennig, K. W. 2002. Infectious triggers for inflammatory neurological diseases. *Nat. Med.* 8:455.
6 Falcone, M. and Sarvetnick, N. 1999. Cytokines that regulate autoimmune responses. *Curr. Opin. Immunol.* 11:670.
7 Liblau, R. S., Singer, S. M. and McDevitt, H. O. 1995. Th1 and Th2 CD4$^+$ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today* 16:34.
8 Kidd, P. 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* 8:223.
9 Panitch, H. S., Hirsch, R. L., Schindler, J. and Johnson, K. P. 1987. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology* 37:1097.
10 Lassmann, H. and Ransohoff, R. M. 2004. The CD4-Th1 model for multiple sclerosis: a crucial re-appraisal. *Trends Immunol.* 25:132.
11 Black, P. 2001. Why is the prevalence of allergy and autoimmunity increasing? *Trends Immunol.* 22:354.
12 Cunningham, M. W. 2000. Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* 13:470.
13 Rose, N. R. 2001. Infection, mimics, and autoimmune disease. *J. Clin. Investig.* 107:943.
14 Cooke, A., Tonks, P., Jones, F. M. *et al.* 1999. Infection with *Schistosoma mansoni* prevents insulin dependent diabetes mellitus in non-obese diabetic mice. *Parasite Immunol.* 21:169.
15 La Flamme, A. C., Ruddenklau, K. and Backstrom, B. T. 2003. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infect. Immun.* 71:4996.

16 Zaccone, P., Fehervari, Z., Jones, F. M. *et al.* 2003. *Schistosoma mansoni* antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *Eur. J. Immunol.* 33:1439.
17 Sewell, D., Qing, Z., Reinke, E. *et al.* 2003. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. *Int. Immunol.* 15:59.
18 Pearce, E. J., Caspar, P., Grzych, J. M., Lewis, F. A. and Sher, A. 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.* 173:159.
19 Sewell, D. L., Reinke, E. K., Co, D. O. *et al.* 2003. Infection with *Mycobacterium bovis* BCG diverts traffic of myelin oligodendroglial glycoprotein autoantigen-specific T cells away from the central nervous system and ameliorates experimental autoimmune encephalomyelitis. *Clin. Diagn. Lab. Immunol.* 10:564.
20 Martins, T. C. and Aguas, A. P. 1999. Mechanisms of *Mycobacterium avium*-induced resistance against insulin-dependent diabetes mellitus (IDDM) in non-obese diabetic (NOD) mice: role of Fas and Th1 cells. *Clin. Exp. Immunol.* 115:248.
21 Martins, T. C. and Aguas, A. P. 1999. A role for CD45RBlow CD38+ T cells and costimulatory pathways of T-cell activation in protection of non-obese diabetic (NOD) mice from diabetes. *Immunology* 96:600.
22 Borowy, N. K., Sternberg, J. M., Schreiber, D., Nonnengasser, C. and Overath, P. 1990. Suppressive macrophages occurring in murine *Trypanosoma brucei* infection inhibit T-cell responses *in vivo* and *in vitro*. *Parasite Immunol.* 12:233.
23 Sternberg, J. and McGuigan, F. 1992. Nitric oxide mediates suppression of T cell responses in murine *Trypanosoma brucei* infection. *Eur. J. Immunol.* 22:2741.
24 Sternberg, M. J. and Mabbott, N. A. 1996. Nitric oxide-mediated suppression of T cell responses during *Trypanosoma brucei* infection: soluble trypanosome products and interferon-gamma are synergistic inducers of nitric oxide synthase. *Eur. J. Immunol.* 26:539.
25 Mattsson, L., Larsson, P., Erlandsson-Harris, H., Klareskog, L. and Harris, R. A. 2000. Parasite-mediated down-regulation of collagen-induced arthritis (CIA) in DA rats. *Clin. Exp. Immunol.* 122:477.
26 Abdul-Majid, K. B., Jirholt, J., Stadelmann, C. *et al.* 2000. Screening of several H-2 congenic mouse strains identified H-2(q) mice as highly susceptible to MOG-induced EAE with minimal adjuvant requirement. *J. Neuroimmunol.* 111:23.
27 Amor, S., Groome, N., Linington, C. *et al.* 1994. Identification of epitopes of myelin oligodendrocyte glycoprotein for the induction of experimental allergic encephalomyelitis in SJL and Biozzi AB/H mice. *J. Immunol.* 153:4349.
28 Mattsson, L., Lundberg, K., Mussener, E., Jansson, A., Erlandsson Harris, H. and Larsson, P. 2003. Antigen inhibition of collagen-induced arthritis is associated with up-regulation of IL-4 mRNA and induction of Ox40 on T cells in draining lymph nodes. *Clin. Exp. Immunol.* 131:241.
29 Falcone, M. and Bloom, B. R. 1997. A T helper cell 2 (Th2) immune response against non-self antigens modifies the cytokine profile of autoimmune T cells and protects against experimental allergic encephalomyelitis. *J. Exp. Med.* 185:901.
30 Grosskinsky, C. M. and Askonas, B. A. 1981. Macrophages as primary target cells and mediators of immune dysfunction in African trypanosomiasis. *Infect. Immun.* 33:149.
31 Namangala, B., Brys, L., Magez, S., De Baetselier, P. and Beschin, A. 2000. *Trypanosoma brucei brucei* infection impairs MHC class II antigen presentation capacity of macrophages. *Parasite Immunol.* 22:361.
32 Rurangirwa, F. R., Tabel, H., Losos, G., Masiga, W. N. and Mwambu, P. 1978. Immunosuppressive effect of *Trypanosoma congolense* and *Trypanosoma vivax* on the secondary immune response of cattle to *Mycoplasma mycoides* subsp. mycoides. *Res. Vet. Sci.* 25:395.
33 Kaufmann, J., Dwinger, R. H., Hallebeek, A., van Dijk, B. and Pfister, K. 1992. The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in trypanotolerant N'Dama cattle. *Vet. Parasitol.* 43:157.
34 Schleifer, K. W. and Mansfield, J. M. 1993. Suppressor macrophages in African trypanosomiasis inhibit T cell proliferative responses by nitric oxide and prostaglandins. *J. Immunol.* 151:5492.
35 Raes, G., De Baetselier, P., Noel, W., Beschin, A., Brombacher, F. and Hassanzadeh Gh, G. 2002. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. *J. Leukoc. Biol.* 71:597.
36 Beschin, A., Brys, L., Magez, S., Radwanska, M. and De Baetselier, P. 1998. *Trypanosoma brucei* infection elicits nitric oxide-dependent and nitric oxide-independent suppressive mechanisms. *J. Leukoc. Biol.* 63:429.
37 Mabbott, N. A., Coulson, P. S., Smythies, L. E., Wilson, R. A. and Sternberg, J. M. 1998. African trypanosome infections in mice that lack the interferon-gamma receptor gene: nitric oxide-dependent and -independent suppression of T-cell proliferative responses and the development of anaemia. *Immunology* 94:476.
38 Fiorentino, D. F., Bond, M. W. and Mosmann, T. R. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081.
39 Moore, K. W., de Waal Malefyt, R., Coffman, R. L. and O'Garra, A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.* 19:683.
40 Bettelli, E., Das, M. P., Howard, E. D., Weiner, H. L., Sobel, R. A. and Kuchroo, V. K. 1998. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J. Immunol.* 161:3299.
41 Nagelkerken, L., Blauw, B. and Tielemans, M. 1997. IL-4 abrogates the inhibitory effect of IL-10 on the development of experimental allergic encephalomyelitis in SJL mice. *Int. Immunol.* 9:1243.
42 Rott, O., Fleischer, B. and Cash, E. 1994. Interleukin-10 prevents experimental allergic encephalomyelitis in rats. *Eur. J. Immunol.* 24:1434.
43 Cua, D. J., Groux, H., Hinton, D. R., Stohlman, S. A. and Coffman, R. L. 1999. Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. *J. Exp. Med.* 189:1005.
44 Elias, D., Wolday, D., Akuffo, H., Petros, B., Bronner, U. and Britton, S. 2001. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guerin (BCG) vaccination. *Clin. Exp. Immunol.* 123:219.
45 Summers, R. W., Elliott, D. E., Qadir, K., Urban, J. F., Jr, Thompson, R. and Weinstock, J. V. 2003. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. *Am. J. Gastroenterol.* 98:2034.
46 Sewell, D. L., Reinke, E. K., Hogan, L. H., Sandor, M. and Fabry, Z. 2002. Immunoregulation of CNS autoimmunity by helminth and mycobacterial infections. *Immunol. Lett.* 82:101.
47 Barthlott, T., Kassiotis, G. and Stockinger, B. 2003. T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med.* 197:451.