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Research Article

The processing and presentation of endogenous and exogenous antigen by Schwann cells in vitro

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Abstract. The expression of major histocomatibility complex class II in vitro and in vivo by Schwann cells indicates a potential facultative role of Schwann cells in the presentation of antigen to neuritogenic T cells during inflammatory demyelinating neuropathies. Using a T cell proliferation assay, this study demonstrated that processing and presentation of endogenous and exogenous antigen by Schwann cells influences T cell proliferation. Statistical analysis of proliferation and its relation to processing and presentation of antigen by Schwann cells had not been previously addressed. Different combinations of factors including treatment of cultures (untreated, irradiated or fixed), concentration of exogenous antigen (0 or 40 µg/ml), the presence of interferon-γ and the timing of exogenous antigen addition influence the proliferation P_2-specific, non-mammalian protein ovalbumin-specific T cell lines and naive T cells.

Key words. MHC class II; Schwann cells; endogenous; exogenous; T cell.

The efficiency with which Schwann cells process and present major histocompatibility complex (MHC) class II molecules in association with antigen to antigen-specific CD4+ T cells reflects the potential of these cells to act as facultative antigen-presenting cells (APCs) during inflammation. Schwann cells, unlike constitutive APCs such as macrophages [1], B cells [2] and dendritic cells [3] do not readily present antigen-associated MHC class II molecules to CD4+ T cells. Characteristics and factors influencing APC activity include (i) the level of antigen-associated MHC class II molecule expression [3], (ii) cytokine secretion and efficiency of processing and presentation of antigenic peptides [4], (iii) expression of accessory molecules [5] and (iv) the physiological state of cells [6].

The source and efficiency of antigen processing and presentation with MHC class II molecules differ between cell types. The effectiveness of this presentation can influence the ability of cells to elicit an immune response from CD4+ T cells [4]. Other considerations which may influence the pathological role of cells include their anatomical location and their exposure to haemopoeitic cells. There is in vivo and in vitro evidence to show that Schwann cells under particular conditions express MHC class II molecules and can, therefore, behave as facultative APCs [7, 8]. Localised elevation of gamma interferon (IFN-γ), tumour necrosis factor-α (TNF-α) and activated T cells during the inflammation process of inflammatory demyelinating neuropathies (IDNs) provide a scenario for MHC and intercellular adhesion molecule-1 (ICAM-1) up-regulation on Schwann cells during T cell activation in vivo [9, 10]. The sequence of events in IDNs leading finally to the targeting and damage of myelin-forming Schwann cell begs the question of Schwann cell involvement in this process. In this paper, Schwann cell effectiveness at processing and presenting endogenous, digested myelin components, found in abundance during myelin damage in IDNs, and exogenous antigen was determined in terms of T cell stimulation. Questions addressed in this study included the efficiency with which Schwann cells process and present self and non-self

antigen, whether the type of antigen influences efficiency, whether Schwann cells, like constitutive APCs, are capable of stimulating naive T cells in a random pool of lymph node cells and whether Schwann cells effectively stimulate resting autoreactive T cells.

The aim and emphasis of this work was to observe Schwann cell effectiveness at restimulating a P_2-specific T cell line in terms of their ability to process and present endogenous and exogenous P_2 over time. Ovalbumin (OA)-specific CD4+ T cell proliferation by Schwann cells was used as a control to observe the processing and presentation of exogenous OA, a non-mammalian protein. The stimulation of naive T cells, not previously exposed to antigen, by Schwann cells was also observed to determine whether the cells could prime T cells as effectively as professional APCs. The results for Schwann cells were compared to those of professional APCs, thymocytes.

Materials and methods

Source of rat tissue

Inbred Lewis rats from the University of Sydney, Bosch Animal House (University of Sydney Animal Ethics Committee protocol numbers L04/1-92/3/47, L04/1-93/3/572 and L04/5-96/3/2356).

Dissociated Schwann cell cultures

Schwann cells were obtained using sciatic and brachial plexes from newborn rats using an established method [11]. The isolation and culture of Schwann cells followed the method described in Lilje and Armati [12].

Generation of experimental allergic newritis-inducing P_2-specific (OL-1) T cell line

CD4+ T cell cultures were prepared following the method outlined in Lilje and Armati [12]. In brief, P_2-specific T cells were obtained by injecting five 14-week-old male inbred Lewis rats with 0.05 ml bovine P_2 in the hind footpad (kindly supplied by J. Bonner, University of Sydney). Popliteal lymph nodes were harvested on day 13. The cells were incubated with bovine P_2 at 36.5 °C in a 5% CO_2-humidified incubator for 4 days. After 4 days, the cells were maintained in the resting phase by separating live from dead cells using a Ficoll/metrizoate density gradient (Sigma). The live cells were resuspended in RPMI 1640 (Multicel Cytosystems); and washed three times. The cells were then incubated at 36.5 °C for 6–7 days in medium containing 100 ml of RPMI 1640 containing 10% FBS (Multicel Cytosystems); 10% T cell growth factor (TCGF) [12]; 2 mM L-glutamine [Commonwealth Serum Laboratory (CSL)]; 50 IU/ml penicillin G (CSL), 5×10^3 µg/ml streptomycin sulphate (Glaxo), 5×10^{-5} M 2-mercaptoethanol (CSL); 1×10^{-4}% sodium pyruvate (Sigma) and 25 µg amphotericin B (CSL).

The cells were washed two times and restimulated with irradiated (2500 Rad) APCs obtained from the thymus of 8-week-old Lewis rats, in a ratio of at least 1 : 25 T cells to APCs. The T cells were incubated at 36.5 °C in a 5% CO_2- humidified incubator for 3–4 days and after 4 days allowed to rest. Another cycle of stimulation and rest was completed before the cells were either used or frozen.

Determining neuritogenicity of the P_2 specific T cell line by the induction of the animal model of IDNs, experimental allergic neuritis

P_2-specific T cell lines (19.5×10^6/ml) were injected intraperitoneally into five 12-week-old inbred Lewis rats. Animals were then scored clinically for experimental allergic neuritis (EAN) over 13 days on the following scale: +, limp tail; ++, paraparesis; +++, paraplegia, tetraparesis or death [13].

OA-specific T cell line

An OA-specific T cell line, prepared as above, was kindly provided by J. Bonner, University of Sydney.

Flow cytometry

The P_2- and OA-specific T cell lines were characterised by flow cytometry with extracellular markers for the T cell receptor (TcR), CD4 and CD8. After 2 days in rest medium, 10^5 T cells were dispensed into each of six flow cytometry tubes (Disposable Products), washed at 475 g for 7 min with 2 ml of flow cytometry buffer composed of 0.2 g bovine serum albumen (BSA) (Bioscientific) and 1 mM azide (Sigma); in 100 ml PBS. One of the following primary antibodies (15 µl) (all kindly provided by Dr J. Sedgwick, Centenary Institute of Cancer Medicine and Cell Biology) in flow cytometry buffer (30 µl) was added to each flow cytometry tube: W3/25 (mouse anti-CD4), Ox8 (mouse anti-CD8) or R73 (mouse anti-TcR). Ox21 and 45 µl flow cytometry buffer only were used as negative controls. Ox21, an irrelevant antibody with the same isotype as the other primary antibodies (IgG1) was used to determine the specificity of the primary antibodies. Flow cytometry buffer alone was used to determine the specificity of the secondary antibody.

Cells were incubated on ice for 40 min with remixing after 20 min, layered onto FBS and centrifuged at 475 g for 7 min, and washed twice with flow cytometry buffer at 475 g for 7 min. Thirty microlitres of the secondary, FITC anti-mouse antibody (Amersham) in 60 µl of flow cytometry buffer was added to each tube and incubated for 30 min on ice. The cells were layered onto FBS, as above, washed as before, resuspended with 250 µl of flow cytometry buffer and kept on ice until ready for fluorescence analysis with a FACScan (Becton Dickinson) (kindly assisted by J. Webster, Centenary Institute of Cancer Medicine and Cell Biology).

Obtaining naive Lewis rat T cells

Seven, 12-week-old inbred Lewis rats were sacrificed and their popliteal lymph nodes removed aseptically, and a single-cell suspension of lymph node cells was washed with RPMI 1640. The suspension was washed three times at 475 g for 7 min with RPMI 1640. The cells (2×10^5 cells/ml) were incubated in rest medium under standard culture conditions for 2 days. Cells were then washed as above and used in experiments or frozen.

Experimental Design

For group 1, on day 0, Schwann cell cultures (n = 24) containing 2×10^4 Schwann cells each were set up in a 96-well plate. Replicate cultures (n = 4) were treated with complete EMEM containing (i) rIFN-γ (100 IU/ml, Life Technology) and 40 µg/ml exogenous P_2 and (ii) 40 µg/ml exogenous P_2.

The remaining cultures (n = 16) were incubated in complete EMEM with (n = 8) or without (n = 8) rIFN-γ.

Two further sets of replicate cultures (groups 2 and 3) were set up as above in separate 96-well plates.

All cultures were incubated for 3 days after dissociation, followed by washing with DPBS three times. At each stage of the experiment, the Schwann cell cultures were observed to confirm no fibroblast contamination. Group 1 cultures were left untreated, group 2 cultures were irradiated with 2500 Rad and group 3 cultures were fixed for 2 h at room temperature with 2.5% paraformaldehyde in DPBS. Irradiated and fixed cultures were used to observe the treatments effect on processing and presentation of antigen and interaction with T cells.

All cultures were washed and refed with 200 µl of T cell-stimulating medium containing 2×10^5 P_2-specific T cells. Cultures previously treated with IFN-γ had fresh IFN-γ added and cultures previously treated with P_2 were treated with the same dose of P_2.

The peripheral nerve cultures in groups 1, 2 and 3 treated with rIFN-γ but not P_2 on day 0 (n = 8) were split into replicate cultures (n = 4) and treated as follows: (i) EMEM plus rIFN-γ alone or (ii) 40 µg/ml exogenous P_2.

The peripheral nerve cultures in groups 1, 2 and 3 not treated with rIFN-γ or P_2 on day 0 (n = 8) were also split (n = 4) and treated as above minus rIFN-γ. Groups 1, 2 and 3 cultures were incubated for a further 3 days before analysis. Each experiment consisting of group 1, 2 and 3 was replicated three times.

Controls

Professional APCs were used as a basis of comparison with Schwann cells to indicate optimum T cell responsiveness to antigen presentation. On day 3 a positive control consisting of replicate cultures (n = 4) containing 200 µl stimulation medium with 2×10^4 irradiated (2500 Rad) thymocytes, 2×10^5 P_2-specific T cells and 20 µg/ml of exogenous P_2 minus Schwann cells was set up in each 96-well plate. Negative controls consisting of replicate cultures (n = 4) containing 200 µl stimulation medium with 2×10^5 P_2-specific T cells and 20 µg/ml of exogenous P_2 only were also set up in each 96-well plate.

Proliferation assay

The CellTiter 96TM Non-Radioactive Cell Proliferation Assay (Promega) was used to determine the proliferation of T cells. The method described in Lilje and Armati [12] was used to quantify proliferation. The intensity of the colorimetric reaction, an index of T cell numbers, was then read and the normalised absorbance readings were recorded using the microplate reader (Model 450, Bio-Rad).

Statistical analysis

Cochran's test showed that there were heterogeneous variances in all sets of data; however, the design of the experiments with multiple replicates validated the use of four- and five-factor analysis of variance (ANOVA) [14]. The factors were (i) number of replicate experiments, (ii) treatment of cultures (untreated, irradiated or fixed), (iii) concentration of exogenous antigen (0 or 40 µg/ml), (iv) plus/minus IFN-γ, with the additional factor for five-factor ANOVA of (v) time of exogenous antigen addition, that is either 3 days before or at the same time as T cells. Significant interactions between factors are indicated by the probability (p) being less than the 0.05 significance level. Following ANOVA, the relationship of treatments within factors and between factors was identified by comparing the replicate means for each treatment combination using Student-Newman-Keuls test.

Results

The P_2-specific T cell line induced EAN, indicating neuritogenicity, and were primarily CD4+

Four out of five adult Lewis rats injected with 19.5×10^6/ml P_2-specific T cells developed mild EAN by day 13 (table 1).

Flow cytometry showed that the P_2-specific T cell line was 100% TcR+, 61% CD4+ and 37% CD8+. The unlabelled cells appeared to be dead or remnant thymocytes. Ninety percent of the OA-specific T cells were TcR+, 62% CD4+ and 28% CD8+. Despite the presence of CD8+ T cells in the P_2 and OA-specific T cell lines, there was no observed cytotoxic affect of these CD8+ T cells on Schwann cells treated with the T cell lines.

Schwann cells induced significantly higher proliferation of P_2-specific T cells than irradiated or fixed Schwann cells plus or minus exogenous P_2

Four-factor ANOVA showed that there was a significant interaction between Schwann cell treatment and concen-

Table 1. Summary of P_2-specific T cell induction of EAN.

	Day		
	1 to 7	8 to 11	12 to 13
P_2	–	+	++

+, limp tail; ++, paraparesis; +++, paraplegia, tetraparesis or death.

tration of exogenous P_2 ($F_{4,4} = 22.78$, $p < 0.01$). Neither 0 or 40 µg/ml P_2 (78.9 ± 5 and 80.9 ± 18.7, respectively) nor preincubation with IFN-γ with 0 or 40 µg/ml P_2 (80.7 ± 3.8 and 86.4 ± 6.5) affected the significant T cell proliferation induced by untreated Schwann cells (fig. 1A). Preincubation of irradiated and fixed Schwann cells with IFN-γ with 0 or 40 µg/ml P_2 did not statistically influence T cell proliferation (63.8 ± 10.7 and 54.2 ± 5.5, respectively; however, the presence of 40 µg/ml P_2 before irradiation or fixation enhanced T cell proliferation (fig. 1B,C) with IFN-γ (79.8 ± 16.7 and 86.7 ± 4.0, respectively) and without IFN-γ (69.7 ± 15.6 and 81.1 ± 7.6, respectively). The baseline comparison of normalized P_2-specific T cell proliferation without Schwann cells or thymocytes (21.4 ± 4.6) (fig. 2) was significantly lower than P_2-specific T cells incubated with Schwann cells (fig. 1A–C).

Schwann cells induced OA-specific T cell proliferation only when exogenous OA antigen, a non-neural antigen, was added

Four-factor ANOVA indicated significant interaction between Schwann cell treatment, concentration of exogenous OA and the presence on absence of IFN-γ ($F_{4,8} = 20.65$, $p < 0.01$). Untreated Schwann cells plus 40 µg/ml OA with and without IFN-γ induced significant T cell proliferation (92.4 ± 5.4 and 86.2 ± 21.1, respectively) in comparison to cultures not exposed to OA (53.7 ± 9.5 and 40.1 ± 5.9, respectively) (fig. 3A). Preincubation with IFN-γ significantly increased the level of T cell proliferation further with untreated Schwann cells in the presence of 40 µg/ml OA (92.4 ± 5.4) but not 0 µg/ml OA (53.7 ± 9.5). Although irradiated or fixed Schwann cells plus OA also induced significant T cell proliferation with IFN-γ (91.3 ± 4.2 and 90.1 ± 9.2, respectively) or without IFN-γ (54.5 ± 7.1 and 70.1 ± 11.1, respectively) (fig. 3B, C), the levels were always lower than for T cell proliferation with untreated Schwann cells plus OA. Proliferation of OA-specific T cells by irradiated and fixed Schwann cells minus OA (32.5 ± 4.8 and 24.5 ± 2.4, respectively) was comparable to the negative control (fig. 2).

Longer exposure of untreated Schwann cells to OA significantly enhanced OA-specific T cell proliferation

Five-factor ANOVA showed that the time at which 40 µg/ml OA was added significantly influenced OA-specific T-

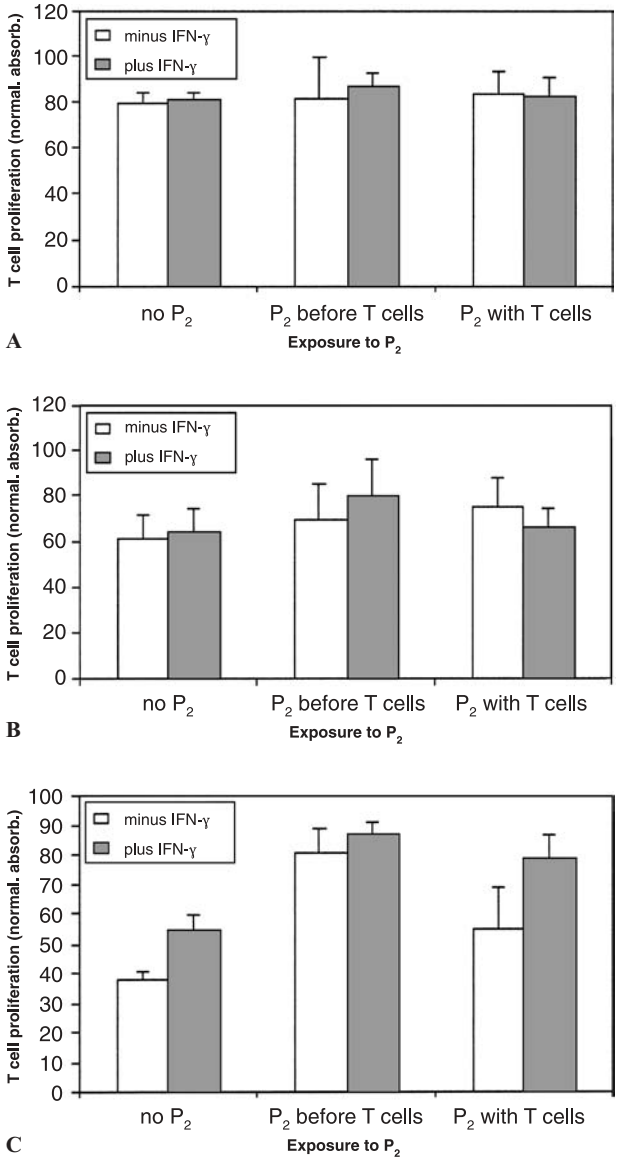

Figure 1. (*A*) Untreated Schwann cells were either incubated or not with rat INF-γ. Cultures were then exposed either to no exogenous P_2, 40 µg/ml P_2 3 days before P_2-specific T cells or 40 µg/ml P_2 with P_2-specific T cells. All proliferation responses were around the normalized T cell proliferation mean of 80 units. (*B*) Irradiated Schwann cells were either incubated or not with rat INF-γ. Cultures were then exposed either to no exogenous P_2, 40 µg/ml P_2 3 days before irradiation and P_2-specific T cell addition or 40 µg/ml P_2 after irradiation and with P_2-specific T cells. The addition of exogenous P_2 3 days before irradiation plus IFN-γ had a normalized T cell proliferation mean of 80 units. Overall, proliferation without exogenous P_2 or exogenous P_2 after irradiation had a normalized T cell proliferation mean of 10–20 units less than untreated results. (*C*) Fixed Schwann cells were either incubated or not with rat INF-γ. Cultures were then exposed either to no exogenous P_2, 40 µg/ml P_2 3 days before fixation and P_2-specific T cell addition or 40 µg/ml P_2 after fixation and with P_2-specific T cells. The addition of exogenous P_2 3 days before fixation plus or minus IFN-γ had a normalized T cell proliferation mean of 80–90 units. The normalized T cell proliferation mean without exogenous P_2 or exogenous P_2 after fixation was 20–40 units lower than untreated results, except for exogenous P_2 after irradiation plus IFN-γ which had a normalized T cell proliferation mean of 86.7 ± 4.

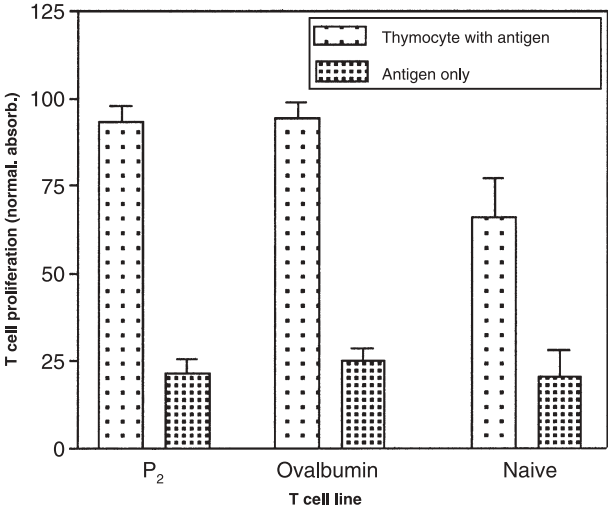

Figure 2. Thymocyte cultures were exposed to exogenous 40 µg/ml P_2 for P_2-specific T cells and naive T cells or 40 µg/ml OA for OA-specific T cells 3 days before treatment (untreated, irradiated and fixed). Normalised T cell proliferation means for P_2- and OA-specific T cells were about 90 units, while for naive T cells, the mean was about 70 units. All normalised T cell proliferation means were low in the presence of exogenous antigen alone (20–25 units).

cell proliferation ($F_{1,1} = 0.93$, $p < 0.05$). OA-specific T cell proliferation was significantly higher for Schwann cells exposed to 40 µg/ml OA 3 days before T cell addition compared to OA added at the same time as T cells with IFN-γ (92.4 ± 5.4 and 40.4 ± 4.9, respectively) or without IFN-γ (86.3 ± 21.1 and 37.8 ± 4.1, respectively) (fig. 3A–C).

Untreated Schwann cell stimulation of naive T cell proliferation was lower in comparison to P_2- or OA-specific T cell lines

Five-factor ANOVA showed that exposure time to P_2 significantly influenced proliferation of naive T cells ($F_{1,1} = 64.93$, $p < 0.05$). The addition of P_2 3 days before naive T cells resulted in significantly higher T cell proliferation than Schwann cell cultures exposed to P_2 at the same time as T cells (36.3 ± 2.9 and 32.1 ± 2.7, respectively). IFN-γ did not affect T cell proliferation for cultures exposed to P_2 3 days before or at the same time as naive T cells (31.9 ± 2.4 and 35.7 ± 6.4, respectively). ANOVA showed that Schwann cell treatment and concentration of exogenous P_2 significantly influenced naive T cell proliferation ($F_{2,2} = 15.93$, $p < 0.05$). Fixed Schwann cells significantly reduced T cell proliferation. Overall, T cell proliferation was only 10–20% above the negative-control level (fig. 4A–C).

The T cell lines, except for naive T cells, were equally responsive to stimulation by thymocytes

P_2- and OA-specific T cell lines in T cell stimulation medium plus respective antigens were equally responsive

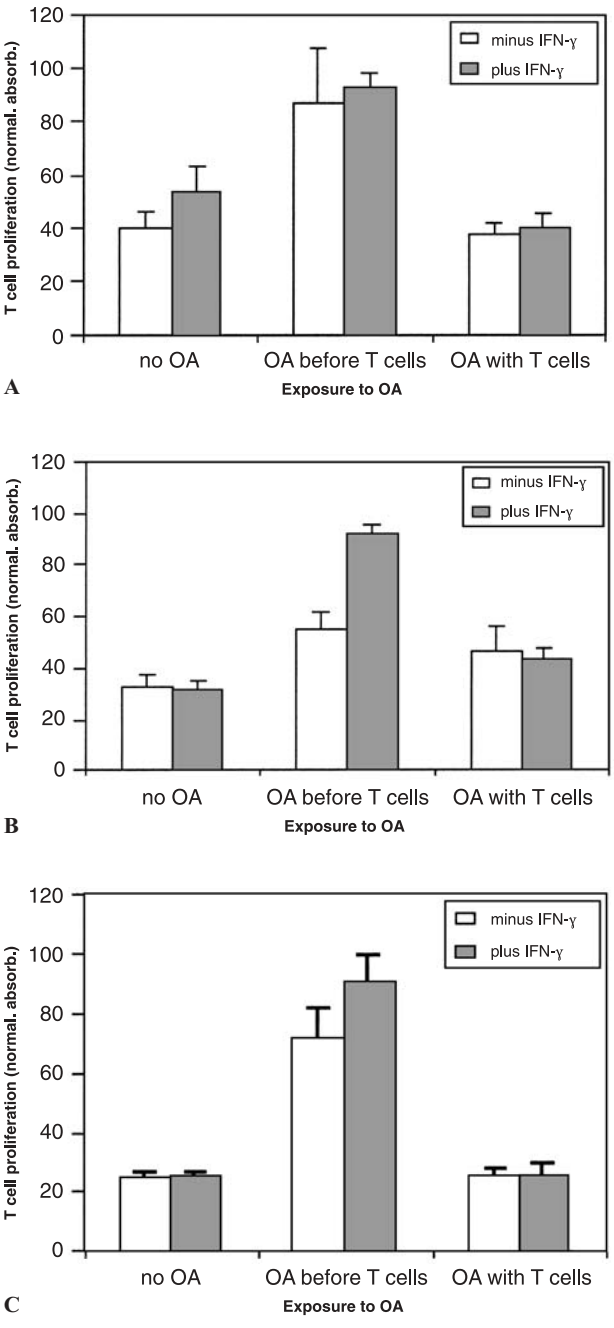

Figure 3. Schwann cells were either incubated or not with rat IFN-γ. Cultures were then exposed either to no exogenous OA, 40 µg/ml OA 3 days before treatment [untreated (*A*) irradiated (*B*) and fixed (*C*)] and OA-specific T cell addition, or 40 µg/ml OA after treatment and with OA-specific T cells. The addition of exogenous OA 3 days before for all Schwann cell treatments had a normalized T cell proliferation mean of 60–90 units. Cultures not exposed to OA or exposed with T cells after treatment had a normalized T cell proliferation mean ranging from 20 to 50 units.

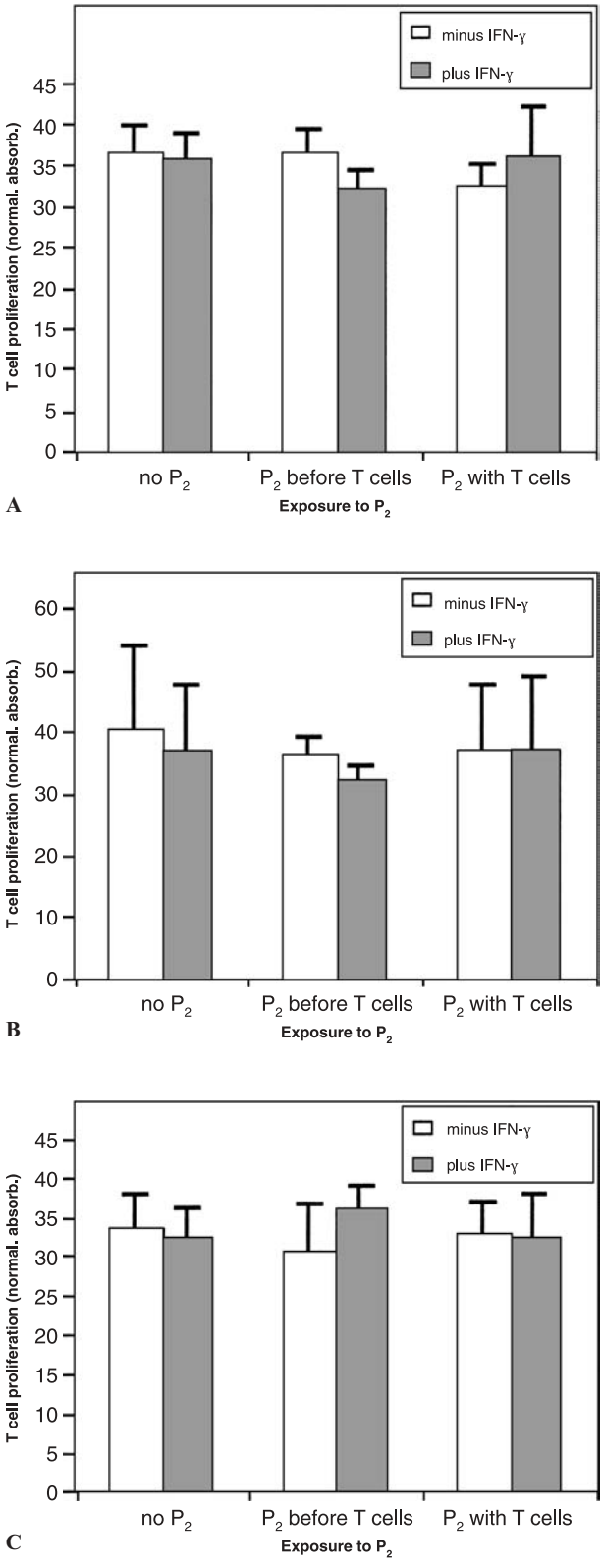

Figure 4. Schwann cells were either incubated or not with rat IFN-γ. Cultures were then exposed either to no exogenous P_2, 40 µg/ml P_2 3 days before treatment [untreated (*A*) irradiated (*B*) and fixed (*C*)] and naive T cell addition or 40 µg/ml P_2 after treatment and with naive T cells. All normalized T cell proliferation means ranged between 30 and 40 units.

to presentation of antigen by thymocytes (94.7 ± 4.3 and 95.6 ± 3.9, respectively). The proliferation levels were higher than those induced by Schwann cells (10%). The same T cell lines in stimulation medium with their respective antigen but minus thymocytes (21.4 ± 4.6 and 25.1 ± 3.6, respectively) had proliferation levels 75% lower than T cells with thymocytes. Overall, proliferation of P_2-specific T cell proliferation by Schwann cells was 5–10% higher than that for the OA-specific T cell line (fig. 2).

Naive T cell proliferation by thymocytes (62.8 ± 8.7) was significantly lower (20–30%) than P_2- and OA-specific T cell lines generated in this study.

Discussion

Activation appears to be critical for migration and interaction of lymphocytes during inflammation (Cross et al., 1990). Molecular mimicry provides one possible explanation for the activation of autoreactive T cells and the initiation of IDNs. The exposure of autoreactive T cells to homologous antigenic determinants of bacterial or viral pathogens most likely occurs in the circulatory system where professional APCs circulate regularly. The results of this study provide further support for this possibility by showing Schwann cell ineffectiveness at priming naive T cells. Schwann cell proliferation of naive T cells was 20–30% lower than that of the antigen-specific T cell lines used in this study. Sedgwick et al. [16] made similar observations for astrocytes and demonstrated that there was limited priming and proliferation of naive T cells. The findings of this study, like those obtained by Sedgwick et al. [16], suggest that such participation of glial cells in IDNs would be secondary in enhancing, perpetuating or suppressing the disease process. Interaction between Schwann cells and activated T cells may enhance Schwann cell presentation of autoantigen and cytokine secretions that may contribute to the proliferation of T cells characteristic of early inflammation. In this study, T cell proliferation was enhanced in the presence of Schwann cells in comparison to T cells not exposed to either Schwann cells or professional APCs (thymocytes).

Unlike P_2, OA is a non-mammalian protein. Processing and presentation would therefore require the antigen to be internalised, transported to endosomes, associated with MHC class II molecules and presented on the cell surface [2, 17, 18]. Such processing would therefore be slower than for endogenous antigens. This is consistent with findings that untreated, irradiated or fixed Schwann cells exposed to OA 3 days before T cell addition were more effective at stimulating an OA-specific T cell response than cultures exposed to OA contemporaneously with the T cells. Similar findings have been reported for B cells, which are professional APCs [19]. B cells transfected

with recombinant vectors coding for hen egg lysozyme and haemagluttinin processed and presented these endogenous antigens more effectively than the corresponding exogenous antigen, because of the partially digested state of the endogenous antigen and the presence of MHC class II precursors [19]. The processing and presentation of endogenous antigen is more efficient than exogenous antigen for various cell types [20].

Reduced stimulation of T cell proliferation by irradiated and fixed Schwann cells compared to untreated Schwann cells indicates the importance of activities such as (i) transcription and translation of MHC class II α and β subunits [21], (ii) association of subunits with the invariant chain in the endoplasmic reticulum [22] and (iii) disassociation from the invariant chain to allow antigen/MHC class II complex formation [23] in the late endosome/dense endocytic compartment [24] during antigen presentation.

Irradiation, as carried out in these experiments, inhibits functions including transcription and translation [25]. Antigen presentation by irradiated Schwann cells may therefore be primarily due to synthesis of MHC class II subunits and the processing and presentation of antigen occurring prior to irradiation. Exposure to exogenous antigen and/or IFN-γ 3 days prior to irradiation or fixation may up-regulate the processing and presentation of antigen by Schwann cells during the first 3 days of incubation. This up-regulation would account for the higher proliferation levels in comparison to Schwann cell cultures exposed to exogenous antigen after irradiation or fixation.

The effectiveness of the synthesis, processing and presentation of antigen/MHC class II complexes is more dramatically highlighted by the fixation of Schwann cells. Fixation results in the immobilisation of inter- and intracellular activity, including transcription and translation. No significant difference in T cell proliferation was observed when P_2 was added before or after fixation of Schwann cells. Fixed endothelial cells and monocytes, like Schwann cells [8], retain some APC function associated with their expression of MHC molecules and costimulatory molecules, such as LFA-1 and CD2 at the time of fixation [26]. Because fixed cells cannot process or present antigen, any T cell interaction and proliferation will occur with the antigen/MHC class II complexes expressed at the time of fixation [18]. There was no significant difference in the proliferation of P_2-specific T cells by fixed Schwann cells in the presence or absence of exogenous antigen added at the same time or 3 days before T cells, providing further evidence for endogenous rather than exogenous antigen presentation.

The relative reduction in T cell proliferation in the presence of irradiated or fixed APCs also reinforces the importance of dynamic intercellular responses and accessory signalling between T cells and APCs during activation. Dynamic intercellular responses include changes in the distribution of ligands on opposing cell surfaces [26] or transient increases in the avidity of LFA-1/ICAM-1 [27].

Results from this study provide the first quantitative findings that Schwann cell presentation of endogenous antigen can restimulate resting antigen-specific T cell lines. Furthermore, the findings indicate that endogenous antigen, such as the myelin component P_2, plays an active role in this restimulation. Myelin is regularly degraded by Schwann cells and could provide a neuritogenic source during IDNs [28]. In terms of APC-like activity, these results indicate the importance of cell contact during antigen presentation by Schwann cells to autoreactive CD4+ T cells. T cell proliferation by Schwann cells was not always dependent on the presence of exogenous IFN-γ, indicating the possibility that (i) the CD4+ T cell lines used are probably Th1 in phenotype and/or (ii) Schwann cells are responsive to physiological levels of cytokines in vitro. This may have significant implications in terms of the potential effects of localised concentrations of inflammatory cytokines on Schwann cells during IDNs. These in vitro observations of Schwann cell responsiveness to antigen, cytokines and T cells suggest a potential contribution of Schwann cells in the pathogenesis of IDNs.

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