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Down modulation of MHC surface molecules on B cells by suppressive immune complexes obtained from chronic intestinal schistosomiasis patients

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Abstract

Granulomatous inflammation around parasite eggs is the prominent lesion in human schistosomiasis. Studies have suggested the involvement of a series of suppressive mechanisms in the control of this reaction, such as macrophages, cytokines, idiotipic interactions and immune complexes (IC). The studies examine the role of IC obtained from chronic intestinal schistosomiasis patients (ISP) in the reactivity of peripheral blood mononuclear cells (PBMC). The results have shown that these immune complexes are able to suppress cell reactivity by inducing an increase in the production of soluble mediators such as prostaglandins and IL-10. To gain a better understanding of how this suppression occurs the present study examines the phenotypic pattern of PBMC after immune complex treatment in cell proliferation assays. These data show that cultures including immune complex present a higher percentage of B lymphocytes in which a lower expression of a MHC-class II gene product, HLA-DR was detected. This altered expression of the HLA-DR molecule on B lymphocytes after IC treatment suggests a novel mechanism for the suppression observed, that is, IC might decrease the antigen-presenting function of B lymphocytes.

Keywords: Schistosoma; Immune complexes; B cells; Antigen-presenting

1. Introduction

Human schistosomiasis is a chronic infection that is caused in the overwhelming majority of cases by one of three species of parasitic helminths: (1) *Schistosoma mansoni;* (2) *S. haematobium;* or (3) *S. japonicum* [1]. The granulomatous lesions in the liver, which consist mostly of eosinophils, macrophages, fibroblasts and lymphocytes, constitute a primary pathological feature of schistosomiasis. These granulomas can evolve into fibrous scars and eventually contribute to widespread fibrosis, portal hypertension and varicocele formation [2]. The development of severe pathology is related to the intensity of infection and it appears to result from an inappropriate host immune response to egg Ag. However, in most patients the immune system develops a series of immunomodulatory processes and a discrete response to egg antigens is observed. These processes have been studied in human schistosomiasis by evaluations of in vitro cell reactivity including cell proliferation and granuloma formation assays [3–5].

In this context, it has been shown that IC isolated from sera of chronic intestinal schistosomiasis patients (ISP) can have an inhibitory effect on in vitro granuloma reaction and cell proliferation to *S. mansoni* anti-

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gens [5–8]. The studies have shown the involvement of soluble mediators such as prostaglandin E series [7] and IL-10 in these mechanisms, accompanied by a decrease in TNF-α an important cytokine involved in granuloma formation [8]. In order to better understand the role played by IC in the suppression of cell reactivity, additional studies concerning the phenotypic characterization of PBMC obtained from schistosomiasis patients were developed. These studies aimed to analyse the influence of IC on the cell phenotype and the expression of costimulatory molecules involved in the reactivity of these cells. No difference was detected in the expression of T lymphocytes and of their subpopulations $CD4^+$ and $CD8^{bright}$ T cells from IC-treated cultures when compared to non-treated cultures. The expression of CD28, a costimulatory molecule involved in the activation of T lymphocytes, was also the same on non-treated and IC-treated cells. Interestingly, a higher percentage of B lymphocytes in IC-treated PBMC were detected but a lower expression of HLA-DR by these cells. This altered expression of HLA-DR on B lymphocytes after IC treatment suggests one of the mechanisms for the suppression observed is through the decrease of the antigen-presenting function of B lymphocytes.

2. Materials and methods

2.1. Study population

Chronic intestinal schistosomiasis patients (ISP) were selected based on clinical and parasitological stool examination for the presence of *S. mansoni* eggs in Fundação Nacional da Saúde, Santa Luzia, MG, Brazil. The patient protocols used throughout this study were approved by the human subject ethics committee in Brazil.

2.2. Isolation of IC

The ISP sera diluted 1/8 were precipitated by using polyethylene glycol 6000 (PEG, Sigma P-2139) at a final concentration of 4%. This mixture was incubated overnight at 4°C, followed by centrifugation at 2500 rpm for 30 min. PEG-induced precipitates were resuspended in PBS to the original sera volume and the quantity of protein was determined by Bradford Method [9]. In the following assays human schistosomiasis PBMC were treated with 125 μg/ml of IC [7].

2.3. Cell preparations

The PBMC were isolated from heparinized blood by Ficoll-diatrizoate density gradient centrifugation (LSM, Organon Teknika, Charleston, SC) as previously described [5]. These cells were cultured in RPMI medium containing 1.6% L-glutamine, 300 U/ml of penicillin, 0.3 mg/ml of streptomycin, 0.05 mg/ml of gentamicin and 10% heat inactivated AB + human serum (RPMI 10% AB) in 96 and 24-well flat-bottomed tissue culture plates (3×10^5 and 1×10^6cells/well, respectively). Every experimental group was set up in triplicate and maintained at 37°C in an atmosphere of 5% CO_2 in air.

2.4. S. mansoni antigen preparations

Adult worms were obtained from BALB/c mice infected 7–8 weeks previously with 200 cercariae by mesenteric vein perfusion [10]. Parasite eggs were isolated from livers of infected mice by homogenization of livers in 1.7% NaCl in 0.15 M phosphate-buffered saline (PBS), pH 7.4 followed by differential sieving, repeated sedimentation and low-speed centrifugation to remove all tissue proteins. Both of them were homogenized in a tissue homogenizer with a teflon pestle [11]. The crude homogenate obtained from eggs or adult worms was centrifuged at $100000 \times g$ for 2 h for the preparation of soluble egg antigen (SEA) and soluble worm antigen preparation (SWAP), respectively. The supernatant was collected, sterile filtered and frozen in aliquots in a -70°C freezer until needed. Protein content was determined using the Bradford Method [9].

2.5. Cell proliferation assay

The PBMC obtained from ISP were cultured in 96 or 24-well flat-bottomed plates in RPMI 10% AB. These cultures were performed with medium alone (CONT), with 25 μg/ml of soluble egg antigen (SEA) or of soluble worm antigen preparation (SWAP). As positive controls cells were stimulated with 10 μg/ml of phytohemaglutinin A (PHA-P, Sigma, St. Louis, MO). For the last 18 h of incubation cells were pulsed with 0.5 μCi/well of tritiated thymidine (ICN Biomedicals, Irvine, CA). On day 5 after culture initiation cells were harvested onto glass fiber paper and incorporation was determined using a β scintillation counter.

2.6. Flow cytometry

Antibodies to cell surface markers were used for phenotypic analysis of peripheral blood leukocytes. The PBMC obtained by Ficoll-diatrizoate centrifugation or cultured for 5 days were washed with RPMI and incubated in 96-well U-bottomed tissue culture plates with a mixture containing FACS buffer and human specific antibodies conjugated to fluorescein isothiocyanate (FITC) or to phycoerytrin (PE). To monitor non-specific binding, isotypic matched MoAbs were used as negative controls. In this assay incubation was performed at 4°C in dark for 30 min. The cells were

washed with PBS and resuspended with 200 μl of MACS FACS fix (10 g/l paraformaldehyde, 1% sodium-cacodilate, 6.65 g/l sodium-chloride, 0.01% sodium-azide). Samples were stored at 4°C before cytofluorometric data acquisition, which was followed by computer analysis. Flow cytometric measurements were performed using a Becton- Dickinson FACScan interfaced to a digital Micro HP 9153C with the LYSIS software for storage and analysis of data. A total of 10000 events/tube were collected. Analysis was performed on the total lymphocyte population by establishing appropriate windows on FSC × SSC dot plots. The large blast population gave rise to poorly defined populations due to extremely low number of events and high background staining with isotype controls and specific antibodies and was excluded from the analysis. Cell phenotyping was performed by evaluating the dot plot of FITC and PE spectra.

$$\%\ \text{decrease in HLA} - \text{DR expression} = \frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100$$

2.7. Statistics

Statistical significance was determined by using Student's t-test and significance was determined with the use of P values < 0.05.

3. Results

3.1. Effect of immune complexes on cell reactivity

In this assay the effect of IC on the proliferative response of PBMC obtained from ISP to SEA and SWAP was tested (Table 1). A total of ten of 12 patients analyzed reacted to SEA, while all of them reacted to SWAP. The response to SWAP was significantly greater than the response to SEA. As observed previously, IC from ISP induced a marked suppression of cell proliferation to SEA and SWAP ($P < 0.05$). The suppressive effect of IC on the proliferative response of PBMC varied from 20 to 71% in cells stimulated with SEA and from 23 to 77% in cells stimulated with SWAP. All patients reacted to the mitogen PHA, however it did not detect a significant suppression of cell proliferation when these cells were treated with IC: PHA = 29.138 cpm, PHA + IC = 23.818 cpm.

Table 1
Effect of immune complexes on the proliferative response to *Schistosoma mansoni* antigens

Antigens	Treatment		% Suppression
	NT	IC	
CONT	1.602 ± 162	2.123 ± 235	no
SEA	11.823 ± 2359	7.128 ± 1430	39%
SWAP	52.959 ± 6620	19.684 ± 1962	63%

Proliferative response of PBMC from chronic intestinal schistosomiasis patients (ISP) to *S. mansoni* antigens. A total of 3×10^5 were incubated in 96-well flat-bottomed plates with no treatment (NT) or with 125 μg/ml of IC (IC). These cells were co-cultured for 5 days with medium (CONT) or 25 μg/ml of *S. mansoni* antigens (SEA and SWAP). For the last 18 h of incubation cells were pulsed with 0.5 μCi/well of tritiated thymidine. On day 5 after culture initiation cells were harvested onto glass fiber paper and incorporation was determined using a β scintillation counter. Results are reported as mean cpm ± SE for the patients studied ($n = 8$).

3.2. Phenotypic analysis of total T cells and subpopulations

To evaluate the influence of IC on the phenotypic pattern of PBMC, these cells were stimulated with SEA or SWAP and cultured in the absence (NT) and presence of IC (IC). On day 5 after culture initiation, PBMC were washed and incubated with anti-CD3 FITC-conjugated antibodies, as described previously and the lymphocyte population was selected according to FSC × SSC dot plot distribution. The mean percentage of T lymphocytes ($CD3^+$ cells) on day 5 of the culture was the same in the control and Ag-stimulated cells. Furthermore, IC-treatment did not induce any change in the percentage of $CD3^+$ cells when compared to non-treated cells (NT). The analysis of the $CD4^+$ and $CD8^+$ T cell subpopulations showed that IC-treatment inhibited these cells to the same extent since the proportion among them was maintained in IC-treated cells when compared to control cells (Fig. 1).

3.3. Expression of CD28 in $CD4^+$ and $CD8^{bright}$ lymphocytes

Since no difference was detected in the percentage of $CD4^+$ and $CD8^{bright}$ lymphocytes, it was decided to investigate if the decreased cell reactivity was related to a change in the expression of costimulatory molecules expressed by these cells. For this, the percentage of CD28 on these cells was examined, a molecule that plays a major role in T cell activation through its interaction with the ligand B7 expressed on antigen-presenting cells. Analysis of the expression of CD28 in $CD4^+$ and $CD8^{bright}$ cells showed no difference in its expression in cells treated or not with IC (Fig. 2).

3.4. Percentage of B lymphocytes

In order to analyse the percentage of B lymphocytes ($CD19^+$ cells) after IC treatment, PBMC were washed and incubated with anti-CD19 PE-conjugated antibodies on day 5 after culture initiation and the lymphocyte

population was selected. In this assay a significant increase was detected in the percentage of B lymphocytes after IC treatment in the control and Ag-stimulated cells when compared to non-treated cells (NT) ($P < 0.05$), suggesting that IC induce an increased proliferation of B cells (Fig. 3).

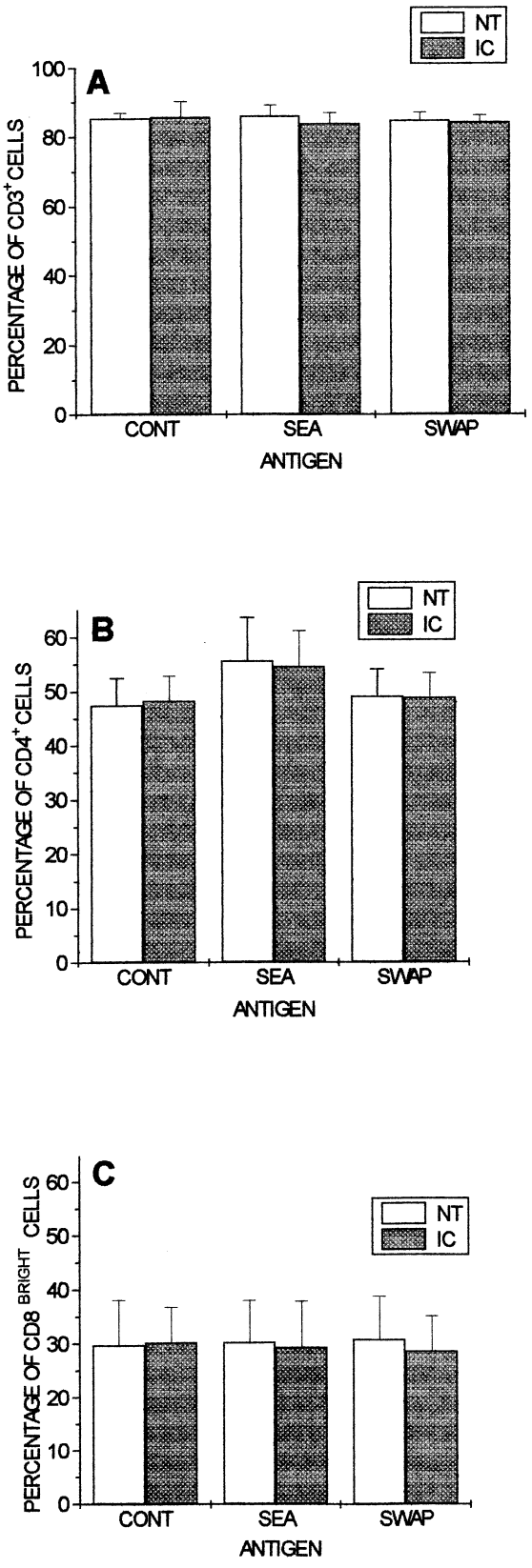

Fig. 1.

3.5. Expression of HLA-DR on B lymphocytes

The B lymphocytes are important antigen-presenting cells for soluble antigens. Moreover, B cells have been shown to increase MHC class II molecules following activation, increasing their presenting ability. The expression of one of these molecules, the HLA-DR was analyzed, in B lymphocytes in order to study the effect of IC on the presentation capability of these cells. Interestingly, a significant mean decrease of 15% was detected in the expression of HLA-DR in cells stimulated with SEA and IC when compared with cells stimulated with SEA in five of six patients studied. This suppression was also observed in cells stimulated with SWAP (12%), in four of seven patients studied (Fig. 4).

4. Discussion

Severe schistosomiasis is a disease characterized by hepatic and intestinal granuloma formation around parasite eggs deposited within the hepatic presinusoidal spaces and the gut. These granulomas constitute the primary pathological feature of schistosomiasis and, in the chronic phase, regulation of this lesion occurs, resulting in smaller anti-egg granulomas [2]. Recent studies have demonstrated that several immunoregulatory mechanisms may be operating in human schistosomiasis. The possible interference of IC with cellular defense mechanisms against *S. mansoni* has been suggested by others [12,13]. Afterwards, studies using in vitro granuloma reaction have shown that IC obtained from sera of ISP are able to suppress PBMC reactivity to polyacrylamide beads conjugated to SEA (PB-SEA) and SWAP (PB-SWAP) [5–8]. This suppressive effect was also observed in the proliferative response of PBMC to parasite antigens [7]. Recent data from the laboratory indicate that IC have the capacity to induce prostaglandin E production by macrophages and thus

Fig. 1. Effect of immune complexes in the expression of $CD3^+$ cells (A) and subpopulations (B, C). A total of 1×10^6 PBMC from ISP were incubated in 24-well flat-bottomed plates with no treatment (NT) or with 125 μg/ml of IC (IC). These cells were co-cultured for 5 days with medium (CONT) or 25 μg/ml of *S. mansoni* antigens (SEA and SWAP). After a 5-day culture PBMC were washed and incubated with antibodies to cell surface markers (CD3, CD4 or CD8). The analysis of specific cell population was performed by establishing appropriate windows on FSC × SSC dot plots. Cell phenotyping was done by evaluating the dot plot to FITC and PE spectra. Results are expressed as the mean ± SE for the patients studied ($n = 5$).

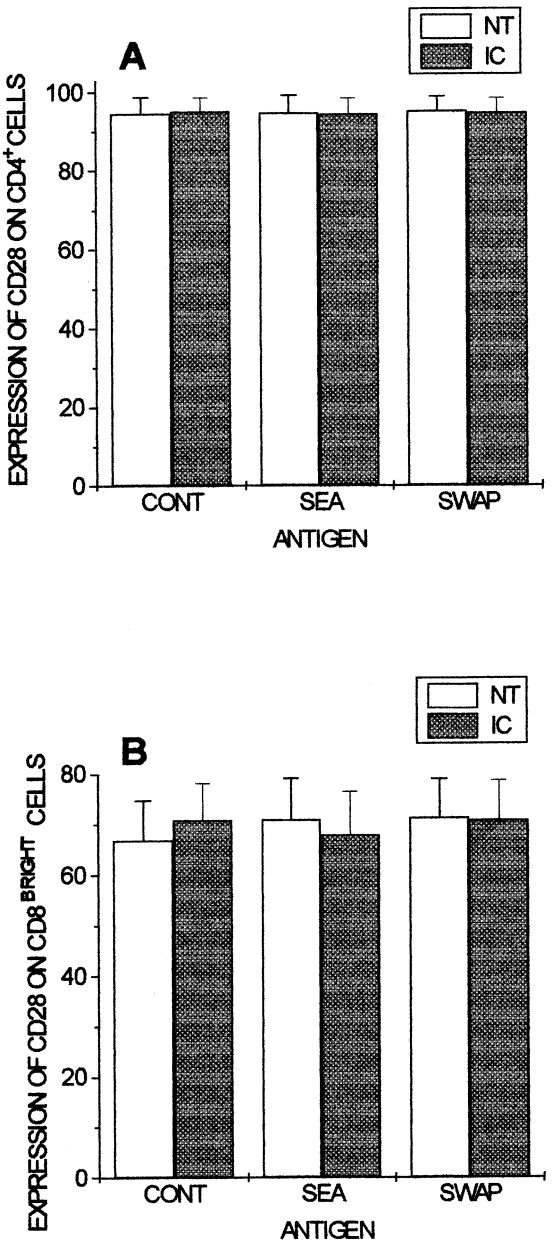

Fig. 2. Effect of immune complexes in the expression of CD28 in $CD4^+$ (A) and $CD8^{bright}$ cells (B). A total of 1×10^6 PBMC from ISP were incubated in 24-well flat-bottomed plates with no treatment (NT) or with 125 μg/ml of IC (IC). These cells were co-cultured for 5 days with medium (CONT) or 25 μg/ml of *S. mansoni* antigens (SEA and SWAP). On day 5 of culture PBMC were washed and incubated with anti-CD4/CD8 FITC and anti-CD28 PE monoclonal antibodies. The analysis of specific cell population was performed as described in materials and methods. Results are expressed as the mean $\pm$ SE for the patients studied ($n = 6$).

inhibit the initial events involved in granuloma formation [6]. Furthermore the involvement of cytokines in this system have been demonstrated [8]. The studies show that IC induce an increase in IL-10 production and a decrease in TNF-α production by mononuclear cells from schistosomiasis patients. However, the characterization of immunoregulatory events induced by IC is still incomplete.

In order to better understand how this suppressive mechanism occurs, the phenotypic pattern of mononuclear cells after immune complex treatment in cell proliferation assays was examined. In this system, no difference was detected in the expression of $CD3^+$ lymphocytes when PBMC were treated with IC as compared to non-treated cells. Furthermore, the ratio among $CD4^+$ and $CD8^{bright}$ cells was maintained showing that IC suppresses their proliferation to the same extent.

In looking for possible mechanisms that may cause IC-induced suppression of cellular reactivity, the expression of costimulatory molecules on the surface of T cells was analyzed. The CD28/B7 interaction has been implicated as a major participant in the primary activation of T cells. In healthy donors, the CD28 marker is expressed by almost 95% of peripheral blood $CD4^+$ cells and by approximately 75% of $CD8^{bright}$ cells. In HIV-infected patients, an altered pattern of CD28 expression on both $CD8^{bright}$ and $CD4^+$ cells has been found, possibly explaining defects in the cytokine pattern and immune response peculiar to these patients [14]. In the system, no difference was detected in the expression of the CD28 marker in $CD4^+$ and $CD8^{bright}$ cells after IC-treatment. Furthermore, no difference was found in the expression of B7 on B cells (data not shown) among not treated and IC-treated cells.

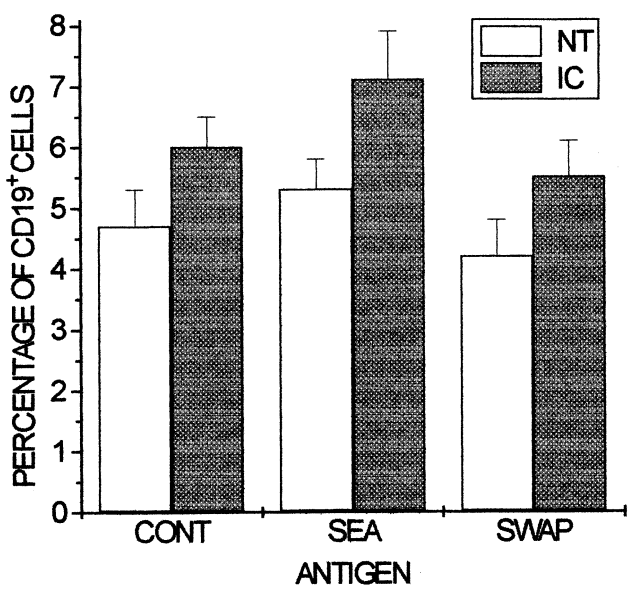

Fig. 3. Effect of immune complexes in the expression of $CD19^+$ cells. A total of 1×10^6 PBMC from ISP were incubated in 24-well flat-bottomed plates with no treatment (NT) or with 125 μg/ml of IC (IC). These cells were co-cultured for 5 days with medium (CONT) and 25 μg/ml of *S. mansoni* antigens (SEA and SWAP). On the day 5 of culture PBMC were washed and incubated with anti-CD19 PE antibody. The analysis of specific cell population was performed as described in Section 2. Results are expressed as the mean $\pm$ SE for the patients studied ($n = 5$).

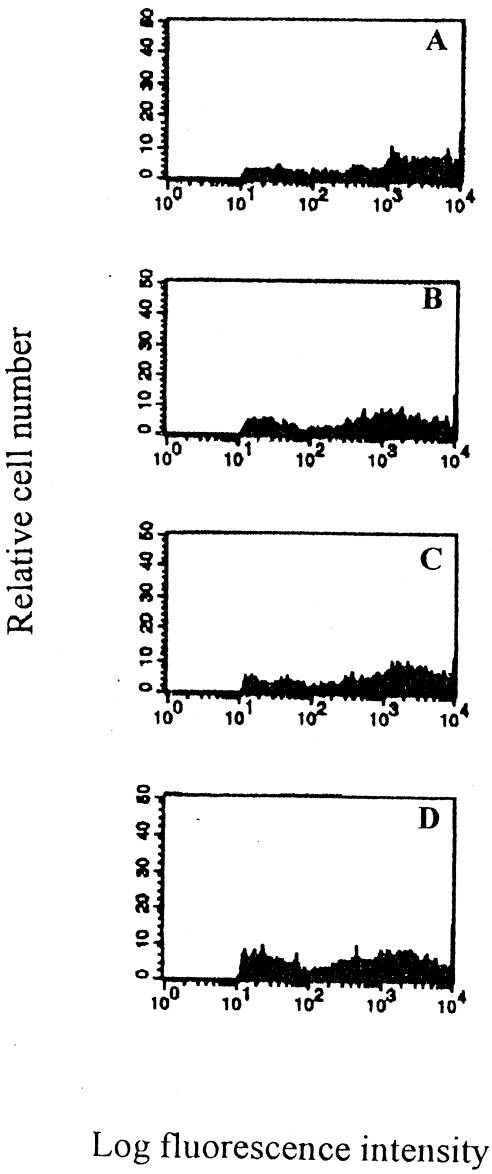

Fig. 4. HLA-DR expression on CD19$^+$ after IC treatment. A total of 1×10^6 PBMC from ISP patients were cultured in 24-well flat-bottomed plates with no treatment (A, C) or with 125 μg/ml of IC (B, D). These cells were co-cultured with SEA (A, B) or SWAP (C, D). After a 5 day culture PBMC were washed and incubated with anti-CD19 FITC and anti-HLA-DR PE antibodies. CD19$^+$-HLA-DR$^+$ cells were gated and analyzed by histogram plot. Mean fluorescence intensity values for the experiment presented were A = 2.438, B = 1.743, C = 2.035 and D = 1.533.

An interesting finding was the detection of increased number of B cells in IC-treated PBMC. The B lymphocytes are present in great amounts near the egg and are responsible for the high level of antibody produced in response to antigens released from the eggs and the formation of immune complexes. These IC are in part eliminated by their ligation to Fc receptors on macrophages [5] which results in an increased production of soluble mediators that are responsible for the suppressive mechanisms observed in the system, that is, prostaglandins and IL-10. The IL-10 has been shown to have a B cell growth-promoting activity in vitro [15] and might be responsible by the increased number of B lymphocytes observed. On the other hand, B cells may function as an additional source of suppressive IL-10.

Furthermore, studies performed in a group of schistosomiasis patients suggest a role for B cells in Ag-specific suppression of cell reactivity induced by IC (not published). These studies have shown that IC obtained from schistosomiasis patients can alter the proliferative response of PBMC to SEA and SWAP differently. In some of these patients it was observed that the IC were able to induce a suppression in the proliferative response to SWAP but not to SEA, while in others the suppression was detected in the response to both antigens. Based on these results it is believed that other mechanisms, besides Fc receptor mediated phagocitosis by macrophages, can be involved in the uptake of IC. These IC might be eliminated by ligation to Ag-specific B cells through the binding to membrane immunoglobulin and Fcγ receptors on B cells simultaneously, possibly inhibiting B cell activation and Ag presentation.

The finding of decreased expression of the MHC-class II gene product, HLA-DR, on the surface of B cells, suggests a mechanism for decreased antigen presenting capacity by these cells. The results show that immune complex-treated B cells present a lower expression of HLA-DR molecule on their surface. This molecule is expressed constitutively on B lymphocytes and, upon up-regulation, it is involved in the presentation of soluble proteins to T lymphocytes. The observed lower expression of HLA-DR on B lymphocytes after IC treatment suggests a novel mechanism for the suppressive activity observed, that is, IC might decrease the antigen-presenting function of B lymphocytes.

In conclusion, the present study clarifies the mechanisms by which IC could be acting to decrease cellular reactivity in human disease and highlights the complexity at this suppression, involving altered cytokine production [8], cellular content and levels of expression of class II MHC.

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