Maternal schistosomiasis alters costimulatory molecules expression in antigen-presenting cells from adult offspring mice

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Highlights

- Study performed with adult offspring born or suckled by schistosomotic mothers.
- Expression of costimulatory molecules on APCs in response to OA was investigated.
- Suckling by infected mothers enabled an improvement antigen presentation by B cells.
- Gestation imprinted in the offspring a weak antigen presentation by APCs.

Graphical Abstract

- A diagram illustrating the experimental setup and outcomes.

Abstract

Adult offspring of Schistosoma mansoni-infected mice showed alterations in immunity to a heterologous antigen, ovalbumin (OA). Prior breastfeeding induced increased production of anti-OA antibodies, while pregnancy impaired it. Here, we investigated the expression of costimulatory molecules on antigen-presenting cells (APCs) of the adult offspring of S. mansoni-infected mothers in response to OA. Newborn mice were divided into three groups: animals Born Infected Mothers (BIM) suckled by non-infected mothers; animals from non-infected mothers Suckled Infected Mothers (SIM); and another group of mice born from and suckled by non-infected mothers (CONTROL). The adult offspring were immunized with subcutaneous OA + adjuvant, and 3–8 days following immunization, double labeling was performed (CD45R/B220 or CD11c and CD80, CD86, CD40 or HLA-DR) on spleen cells. In comparison to the CONTROL group, an early increased frequency of CD40+/CD80+ B cells was observed in SIM mice (p < 0.001), but no alteration of CD11c+ cells was observed. In contrast, in BIM mice, the frequency of CD86+/CD11c+ cells (p < 0.05) and CD40+/CD80+/CD86+ B cells (p < 0.01/p < 0.01/p < 0.05) was drastically reduced. In conclusion, previous suckling by S. mansoni-infected mothers enabled improved antigen presentation by B cells in adult offspring, whereas gestation in these mothers imprinted offspring with weak antigen presentation by APCs during the immune response to a non-related antigen.

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1. Introduction

_Schistosoma mansoni_ infection is highly prevalent in tropical regions that include Northeast and Southeast Brazil (Barbosa et al., 2006). Schistosomiasis is marked by a delayed-hypersensitivity reaction with consequent granulomatous inflammation and fibrosis around the parasites’ eggs in the liver and intestine (Wynn et al., 2004). _S. mansoni_ egg antigens elicit a dominant Th2 response (interleukin (IL)-4, IL-13, IL-10, eosinophilic infiltrated and fibrosis) (Everts et al., 2009; Pearce et al., 1991; Wynn et al., 2004), in addition to regulatory T cell environment (Taylor et al., 2006; Wilson et al., 2007). Infected pregnant women are commonly observed in endemic areas (Friedman et al., 2007). Regarding the influence of maternal schistosomiasis on the immunity of offspring, an attenuation of schistosomiasis symptoms in postnatal infections has been observed (Attallah et al., 2006; Caldas et al., 2008; Lenzi et al., 1987; Othman et al., 2010).

However, very little is known about the effects of maternal _S. mansoni_ infection on the immune response to a heterologous antigen in the adult offspring (Noureldin and Shaltout, 1998). Recently, in an experimental model, we demonstrated the effect of pregnancy and nursing by a _S. mansoni_-infected mother on the immune response to ovalbumin (OA) in adult offspring. Suckling by infected mothers enhanced the OA-specific humoral response in the adult offspring. In contrast, gestation in _S. mansoni_-infected mice impaired the OA-specific humoral immune response in an IL-10-dependent manner (Santos et al., 2010).

The development of a cellular and humoral immune response to protein antigens is triggered by interactions between CD4+ T lymphocytes and antigen-presenting cells (APCs), including dendritic cells (DCs), B lymphocytes and macrophages. Following recognition of the antigen on DCs by naïve T cells, the proliferation and differentiation of the T cells into effector Th cells, as well as the full activation of B lymphocytes, requires the engagement of CD28 on APCs by the CD28 on the T cell and the CD80 (B7.1) and CD86 (B7.2) costimulatory molecules on the APCs ( Kearney et al., 1995; Riha and Rudd, 2010). However, CD80 and CD86 molecules are also ligands of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) on T cells, a molecule that induces an intracellular signal that inhibits T cell activation (Bour-Jordan et al., 2011; Tivol et al., 1995). Therefore, the levels of CD80 and CD86 expressed on APCs could affect the balance between CD28- and CTLA-4-dependent immune outcomes. Additionally, CD40 Ligand (CD40L) on activated T lymphocytes interacts with CD40 molecules on APCs. This CD40:CD40L interaction is necessary for different effector functions; in addition to T cell activation, it improves antigen capture and presenting capacity by APCs and supports antibody isotype switching by B lymphocytes (Ma and Clark, 2009).

In this study, we investigated the expression of costimulatory molecules on APCs from adult offspring of _S. mansoni_-infected mothers to explain the immunosuppressive potential induced by pregnancy as well as the enhancement of antibody production due to previous suckling by these mothers. For this, spleen cells from adult offspring born from or suckled by infected mothers were analyzed for expression of CD40, CD80 and CD86 molecules on CD11c+ cells and cells after OA immunization. Our results showed that gestation in infected mothers impaired costimulatory molecule expression on B lymphocytes (CD45R/B220+) and myeloid APCs (CD11c+), whereas suckling by infected mothers improved the CD40 and CD80 expression on B lymphocytes in response to OA. Thus, maternal schistosomiasis can imprint in the adult offspring an altered immune response to non-parasitic protein antigens.

2. Materials and methods

2.1. _S. mansoni_ infection and adoptive nursing

Four-week-old Swiss webster female mice were infected, subcutaneously, with 20 _S. mansoni_ cercariae, São Lourenço da Mata (SLM) strain (Pernambuco, Brazil). On the 60th day post-infection, estruses were synchronized by administration of 5 i.u. (100 μl) of eCG (equine chorionic gonadotrophin) hormone plus, after 48 h, injection of an additional 5 i.u. (100 μl) of hCG (human chorionic gonadotrophin) (Fowler and Edwards, 1957). The females were caged with male mice at a 1:1 ratio and successful mating was checked by presence of a vaginal plug. The same procedure was performed in non-infected females.

Immediately after birth, the newborns male mice from _S. mansoni_-infected or non-infected mothers were housed in cages with interchanged mothers. After adoptive nursing, offspring mice Born Infected Mothers (BIM) were suckled by non-infected mothers. The offspring of non-infected mothers were Suckled Infected Mothers (SIM). Animals born from non-infected females were also suckled by their own mothers (CONTROL). The mice were housed in the animal care facility at the Aggeu Magalhães Research Center, Oswaldo Cruz Foundation, Recife, PE, Brazil. The animal protocol was approved by the Ethics Committee for Animal Research of the Federal University of Pernambuco.

2.2. Immunization protocol and study groups

Six-week-old male offspring were taken for the experimental and control groups. The mice were immunized, subcutaneously, with 100 μg of ovalbumin (OA) (grade V; Sigma-Aldrich) emulsified in complete Freund’s adjuvant (CFA) (Sigma–Aldrich), at the base of the tail (0.1 ml/animal). Mice were divided into six groups (n = 10): (1) mice BIM + OA; (2) non-immunized mice BIM; (3) mice SIM + OA; (4) non-immunized mice SIM; (5) CONTROL + OA and (6) non-immunized CONTROL.

2.3. Ex vivo staining

Spleens from experimental or control mice collected 3, 5 and 8 days after OA immunization were cut with scissors and left in a plate for 1 h at 37 °C in the presence of collagenase (1 mg/ml, Sigma Chemical Co., St. Louis, MO, USA). The cell suspension obtained was washed in RPMI 1640 (Sigma Chemical) supplemented with 5% fetal calf serum (FCS) (Sigma–Aldrich) 0.1 mM L-glutamine, 0.5 mM 2-mercaptoethanol and 1% penicillin and streptomycin. Double labeling was performed after incubation (4 °C for 30 min) with fluorochrome-labeled antibody solutions at a concentration of 0.5 mg/10^6 cells: PE-Cy5 anti-mouse CD45R/B220 or PE-Cy5 anti-mouse CD11c plus PE anti-mouse CD80, PE anti-mouse CD86, PE anti-mouse CD40 or PE anti-mouse HLA-DR (all from BD Biosciences Pharmingen, San Diego, CA, USA). Immunoglobulin isotype controls conjugated to PE-Cy5 or PE were also used. After staining, the preparations were washed with PBS containing azide (0.1%) and fetal bovine serum (3%). After centrifugation, the cell pellet was resuspended in PBS with paraformaldehyde (0.5%) and maintained at 4 °C until the moment of data acquisition.

2.4. Flow cytometric analysis

Data acquisition was performed using a flow cytometer (FACScalibur (BD-Pharmingen, San Diego, CA, USA)) by collecting a minimum of 10,000 events per sample. The frequency of positive cells was analyzed using the program Cell Quest in two regions. The lymphocyte region was determined using granularity (SSC).
and size (FSC) plot. Antigen-presenting cells were selected based on granularity and expression of CD11c+ or B220+. Limits for the quadrant markers were always set based on negative populations and isotype controls. A descriptive analysis of the frequency of cells in the upper right quadrant (double-positive cells) was performed. To analyze the frequency of cell, statistical differences (\( p < 0.05; p < 0.01; p < 0.001 \)) between groups were assessed using the two-way analysis of variance followed by multiple comparison using the Tukey test. All procedures were repeated three times to evaluate the reproducibility of the results.

3. Results

3.1. CD80, CD86 and CD40 costimulatory expression on myeloid APCs (CD11c+) in adult offspring from S. mansoni-infected mothers

The frequency of CD11c+ cells expressing CD80 and CD86 in spleen cell suspension from mice born from or suckled by S. mansoni-infected mothers was evaluated on days 3, 5 and 8 after immunization with OA. The frequency of these cell subsets was also obtained in non-immunized mice (Fig. 1a). There was a similar frequency of CD86+/CD11c+ cells in the experimental and control groups on days 3 and 5 post-immunization (Fig. 1b and c). In contrast, on the eighth day post-immunization (Fig. 1d), in the mice born from infected mothers (BIM + OA), the frequency of CD11c+ cells expressing CD86 was reduced compared to the CONTROL + OA group and mice suckled in infected-mothers (SIM + OA) (CONTROL + OA = 0.3%; BIM + OA = 0.1%; SIM + OA = 0.27%; \( p < 0.05 \)). No difference in the frequency of CD11c+ cells expressing CD80 was observed among the groups following immunization with OA. There was no difference in CD40+/CD11c+ cells observed among control and experimental groups during the time period following OA immunization.

We found that gestation in or suckling by infected mothers did not alter the intensity of surface HLA-DR on CD11C+ cells (data not shown).

3.2. Pregnancy and suckling in S. mansoni-infected mothers alters the CD80, CD86 and CD40 expression on B cells from adult offspring in response to ovalbumin

The frequency of B cells expressing CD80 and CD86 is depicted in Fig. 2. Control and experimental groups demonstrated a high quantity of CD86+/B220+ cells on the third day following OA immunization (Fig. 2b). The percentage of cells expressing B220 and CD86 was drastically decreased on the eighth day after immunization (Fig. 2d), but the level was much more decreased in the BIM + OA group at the end of the time course in comparison to the CONTROL + OA and SIM + OA groups (CONTROL + OA = 1.2%; BIM + OA = 0.2%; SIM + OA = 1.2%; \( p < 0.05 \)).
Regarding CD80+/B220+ cells, on the third day post-immunization, mice previously suckled in infected mothers showed an increased frequency of double-positive cells (CONTROL + OA = 1.4%; BIM + OA = 1.8%; SIM + OA = 3.5%; \( p < 0.05 \)) (Fig. 2b). There was a high frequency of these cells on the fifth day post-immunization (CONTROL + OA = 12.2%; BIM + OA = 16.1%; SIM + OA = 13.7%) in the control and experimental groups (Fig. 2c). On the eighth day, the frequency of B cells expressing CD80 declined to similar levels in the CONTROL + OA and SIM + OA groups (Fig. 2d). In contrast, at this time point, in animals born from infected mothers, the frequency of D80+/B220+ cells was much decreased compared to CONTROL + OA and SIM + OA mice (CONTROL + OA = 4.2%; BI-M + OA = 0.9%; SIM + OA = 2.9%; \( p < 0.01 \)).

In response to OA immunization, in the SIM + OA group there was a sixfold increase in the frequency of double-positive cells (CONTROL + OA = 1.4%; BIM + OA = 1.8%; SIM + OA = 3.5%; \( p < 0.05 \)) (Fig. 2b). There was a high frequency of these cells on the fifth day post-immunization (CONTROL + OA = 12.2%; BIM + OA = 16.1%; SIM + OA = 13.7%) in the control and experimental groups (Fig. 2c). On the eighth day, the frequency of B cells expressing CD80 declined to similar levels in the CONTROL + OA and SIM + OA groups (Fig. 2d). In contrast, at this time point, in animals born from infected mothers, the frequency of D80+/B220+ cells was much decreased compared to CONTROL + OA and SIM + OA mice (CONTROL + OA = 4.2%; BI-M + OA = 0.9%; SIM + OA = 2.9%; \( p < 0.01 \)).

In response to OA immunization, in the SIM + OA group there was a sixfold increase in the frequency of B220+ cells expressing the CD40 costimulatory molecule as soon as 3 days after OA immunization (Fig. 2b). This also occurred for BIM + OA group, but to a lesser extent (fourfold; CONTROL + OA = 2.5%, BIM + OA = 10.2%, SIM + OA = 15.3%; \( p < 0.001 \)) (Fig. 2b). For all the groups, a high frequency of CD40+/B220+ cells was observed on the fifth day (Fig. 2c), followed by a decrease on the eighth day (Fig. 2d). However, on the last day of the time course, in the BIM + OA group there were much less double-positive cells (0.7%) than in OA-immunized CONTROL (2.7%) and SIM (1.6%) mice (\( p < 0.01 \)).

Neither gestation in nor suckling by infected mothers changed the intensity of surface HLA-DR on B cells (data not shown).

### 4. Discussion

We have demonstrated that gestation in *S. mansoni*-infected mothers influenced the immunity of their offspring on a long-term basis by suppressing the humoral immune response to an unrelated antigen, ovalbumin. In contrast, suckling by infected mothers improved the humoral immune response to this antigen in adult life (Santos et al., 2010). Here, we demonstrate that in adult offspring born from *S. mansoni*-infected mothers, the ability of APCs to present antigen after triggering the immune response to protein antigens was weakened. In contrast, previous contact with the milk of infected mothers enhanced the ability of B lymphocytes to present antigen following OA-specific stimulus.

To continue the previous study design, all offspring were submitted to immunization with OA emulsified in an adjuvant for optimal conditions of Th-cell activation to a soluble protein antigen. In general, approximately 1–7 days are required for an effective immune response to occur in the secondary lymphoid organs, with initial DC contact with naïve Th cells (1–3 days) and later B cell contact with activated Th cells (3–7 days).
dynamics of expression of costimulatory molecules on APCs may differ depending on antigen stimulus (virus, bacteria, parasite infections or protein immunizations) and lead to different immune outcomes. Immunization with OA in adjuvant elicits an effective immune response, with isotype-switched antibodies and cytokines present on the eighth day post-immunization (Souza et al., 2002). Here, analysis of APCs from OA-immunized adult offspring of non-infected mothers showed that CD80 and CD40 molecules on APCs could be better detected on day 5, and CD86 could be best detected on day 3 post-immunization. This last molecule is constitutively expressed and its up-regulation is rapid after antigen recognition (Chen and Flies, 2013; Hathcock et al., 1994).

For adult offspring from S. mansoni-infected mothers (BIM group) immunized with OA, the gestation induced similar frequencies of CD80+ and CD40+/CD11c+ cells as those in offspring born from non-infected mothers. However, there was a striking impairment of CD86+/CD11c+ cells 8 days after OA immunization.

S. mansoni infection induces an unconventional DC activation status. Straw et al. (2003) demonstrated that murine S. mansoni infection induced high CD80 and CD40 expression, but not increased CD86, on purified DCs. There were no alterations on expression of MHC class II, costimulatory molecules, pro-inflammatory, IL-4 and IL-10 cytokines, after exposure to SEA, on DC (Perona-Wright et al., 2006). SEA modulation on DC have been associated with mitogen-activated protein kinase (MAPK) phosphorylation, expression of NF-kB1 and Th2 immune response (Agrawal et al., 2003; Magalhães et al., 2010; Thomas et al., 2003), but in the absence of MyD88, TLR4, TLR2 signalization (Kane et al., 2008; Layland et al., 2005). Meanwhile, TLR2- and TLR-4/-C-type lectins-interaction DC, to lipid and carbohydrate from life cycle stages, respectively (Hokke and Yazdanbakhsh, 2005; Thomas et al., 2003; van Liempt et al., 2007), were able to suppress TLR-induced dendritic cell conventional activation (Kane et al., 2008; van Liempt et al., 2007) with potential anti-inflammatory and IL-10- producing T cells (Layland et al., 2007). Therefore, the lower expression of the CD86 marker in offspring born from infected mice suggests the effect of the previous maternal infection and consequent unusual DCs activation to non-related antigens.

It is known that CD28 binding is more accessible in naive T cells, however CTLA-4 has a stronger binding affinity than CD28 for CD80 and CD86, therefore it can require low density of these molecules. Besides of this, the CD86/CD28 preferential interaction during the DC–T cell initial contact is required for stimulating the immune response, whereas CD80–CTLA-4 interactions induce non-responsiveness in T cells (Plentickx et al., 2011; Wing et al., 2011). In BIM offspring, the APCs may favor signaling by CD80, rather than co-stimulation by CD86, in their interactions with T cells, resulting in impairment in the T-dependent response in these mice (Santos et al., 2010).

Regarding B lymphocytes, BIM offspring demonstrated a very low frequency of CD86+ or CD80+ B cells 8 days after OA immunization. We noticed an increase in the percentage of CD40+ B cells as soon as 3 days after OA immunization, but this increase was followed by a drastic decrease on the eighth day post-immunization compared to the control group. These findings suggest that the appropriate initial activation status of B cells was not maintained in the BIM group. We have demonstrated that the effect of IL-10 in this microenvironment is required for ineffective antibody production in these offspring (Santos et al., 2010). This cytokine can impair the expression of costimulatory molecules on APCs (Moore et al., 2001) and cognate interactions between specific T cells and DCs, as well, as between T and B cells.

Offspring suckled by infected mothers had no significant alteration in the amount of CD11c+ cells expressing costimulatory markers throughout the OA immunization time course. In contrast, B cells from OA-immunized adult offspring that had previous contact with milk from infected mothers showed an upregulation in these markers. These mice demonstrated a rapid increase in the frequency of B cells expressing CD40 and CD80 (third day) followed by a decrease to levels similar to offspring suckled by non-infected mothers (CONTROL + OA). Although CD86 expression was not upregulated in B cells in response to OA, the expression profile for this molecule was similar to that of the control group. Our data indicate that these B cells improve the ligation of CD28 on T cells and CD80/CD86 on B cells (Lumsden et al., 2003). Furthermore, the interaction of CD40/CD40L promotes the maintenance of T cell activation, and it is the main mechanism for T cells to help B cells in antibody production and isotype switching (Hawkins et al., 2013; Lumsden et al., 2003; Ma and Clark, 2009).

Parasite carbohydrate antigens and antibodies are present in milk from S. mansoni-infected mothers (Attallah et al., 2003; Lenzi et al., 1987). Montesano et al. (1999) demonstrated the requirement of anti-SEA antibody during suckling for enhancement of anti-SEA humoral immunity in postnatal murine infections. S. mansoni eggs and worms share with maternal milk the immunomodulatory sugar, Lacto-N-Fucopentose III (LFNP-III) that induces B lymphocyte proliferation (Thomas and Harn, 2004). It is possible that antibodies and immunomodulatory sugars in milk from infected mice imprint a B cell pre-activation status in the descendants, thereby improving antibody production in SIM mice in adult life (Santos et al., 2010).

Maternal exposure to allergens, cytokines or infection, can change the status immune competence of their offspring (Fusaro et al., 2007; Herz et al., 2000; Le Hesran et al., 1997; Lima et al., 2005). Different background of the activation APCs can bias for protective immune response in descendens in the endemic areas for chronic diseases (Arama et al., 2011). In the same way, sensitization in early life can have immunomodulatory effects on the response to heterologous proteins. Here, the expression profile of costimulatory molecules on APCs (low CD86/CD80/CD40 on CD11c+ and B cells) previously “programmed” during gestation in parasitized mother would not promote help from CD4+ T cells and consequent anti-inflammatory effects for allergen and self-antigen in individuals from areas with high prevalence for helminthiasis. In contrast, the suckling in these mother “programmed” the improvement in the intercellular communication pathway for B–T cell and B–DC cell (high CD40/CD80) that favoring antibodies-dependent protection for pathogens and vaccine (Ma and Clark, 2009; Subauste, 2009), as well as, in other fashion the Treg activation (Amu et al., 2010; Harris and Gause, 2011; Zheng et al., 2010).

There are limited data from epidemiological and human interventional trials, in early life, in endemic areas for schistosomiasis. It is true that the extrapolation of data obtained from rodents to humans should be carefully evaluated. Therefore, we believe that further experimental and human studies are required to investigate the possible benefits of the maternal schistosomiasis in primary prevention of allergy, autoimmunity and heterologous infection

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