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Interleukin-10 secreted by B-1 cells modulates the phagocytic activity of murine macrophages *in vitro*

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SUMMARY

As demonstrated previously in our laboratory, B-1 cells migrate from the peritoneal cavity of mice and home to a distant site of inflammation to become macrophage-like cells. However, the influence that these cells might have on the kinetics and fate of the inflammatory process is not known. Considering that macrophages are pivotal in the inflammatory reaction, we decided to investigate the possible influence B-1 cells could have on macrophage activities *in vitro*. Our results show that peritoneal macrophages from Xid mice, a mouse strain deprived of B-1 cells, have higher phagocytic indexes for zymozan particles when compared with macrophages from wild-type mice. Moreover, macrophages from wild-type mice have a lower ability to release nitric oxide and hydrogen peroxide when compared with macrophages from Xid mice. Experiments using cocultures of B-1 cells and macrophages from Xid mice in transwell plates demonstrated that B-1 cells down-regulate macrophage activities. These observations also indicate that this phenomenon is not due to a physical interaction between these two cell populations. As B-1 cells are one of the main sources of interleukin (IL)-10, we demonstrate in this study that adherent peritoneal cells from Xid mice produce significantly less amounts of this cytokine in culture when compared with IL-10 production by cells from wild-type mice. When B-1 cells from IL-10 knock-out mice and macrophages from wild-type mice were cocultured in transwell plates, the phagocytic index of macrophages was not altered demonstrating that B-1 cells can influence the effector functions of macrophages *in vitro* via IL-10 secretion.

Keywords B-1 lymphocyte; down-regulation; IL-10; macrophage; phagocytosis

INTRODUCTION

Although more than 20 years have passed since the discovery of B-1 cells, a large number of studies have been conducted in order to characterize and determine the origin and function of these cells. These efforts have sparked a good deal of controversy and little knowledge has been added regarding the role these cells might have on the modulation of pathological phenomena, such as inflammation.

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It is well established that B-1 cells constitute a minor fraction of the B cell population in spleen and are not detected in lymph nodes of mice. Nevertheless, they represent the main B cell population in the peritoneal and pleural cavities of these animals.[1] They express high levels of surface IgM and low levels of B220 and IgD, but not CD23, whereas conventional B-2 cells express high levels of B220, IgD and CD23 and low levels of IgM.[2] B-1 cells also express low levels of Mac-1, and a subset designated B-1a have intermediate levels of CD5 on their surface.[2–4] However, IgM and CD5 antigens are lost when B-1 cells migrate out of the peritoneal cavity.[4,5]

Almeida *et al.*[5] demonstrated in our laboratory that B-1 cells proliferate in spontaneous stationary cultures of normal adherent mouse peritoneal cells. Additional experiments provided support for this conclusion, because when peritoneal cells from Xid mice were cultivated B-1 cells did not proliferate in the culture medium. As demonstrated in

the literature, Xid mice have impaired production of B-1 lymphocytes.[6] Further, it was also demonstrated that B-1 cells migrate to a non-specific inflammatory focus and differentiate into a macrophage-like cells.[5] Nevertheless, the role these cells might play on the kinetics and fate of the inflammatory response and on parasite infection is not established. However, it has been demonstrated that Xid mice are significantly more resistant to *Trypanosoma cruzi*,[7] *Paracoccidioides brasiliensis* (Godoy *et al*., in preparation) and lymphatic filarial parasite infections.[8] These data support the hypothesis that B-1 cells could down-regulate the efficacy of effector cells, such as macrophages, to eliminate parasites in the inflammatory milieu. In this regard, it is known that B-1 cells produce and utilize interleukin (IL)-10 as an autocrine growth factor[9] and that this cytokine is an important negative regulator of cell-mediated immunity.[10] Further, it has been demonstrated that IL-10 inhibits the generation of reactive oxygen intermediates,[11] impairs antigen-presenting cell function by macrophages[12] and decreases production of cytokines by these cells in response to interferon (IFN)-γ.[13]

Based on these data we decided to investigate the possible influence B-1 cells might have on macrophage functions *in vitro*.

MATERIALS AND METHODS

Mice

Male BALB/c and BALB/c Xid mice, 6–12 weeks old, were obtained from animal facilities from the Department of Immunology, University of São Paulo. C57BL/6 wild-type and C57BL/6 IL-10 knock-out (KO) mice were kindly donated by Professor João Santana da Silva from the Department of Pharmacology, School of Medicine, USP, Ribeirão Preto.

Mice irradiation

Based on evidence that B-1 cells are radiosensitive,[5] mice were lethally irradiated (700 rad) in a Nordion Gammacell, and 2 days later cells from their peritoneal cavities were collected and cultured as described below.

Analysis of peritoneal cell phenotypes

The phenotype of total peritoneal cells obtained from wild-type BALB/c and BALB/Xid, submitted or not to irradiation, was analysed by fluorescence activated cell sorter (FACS). Cells were collected from the peritoneal cavity of the animals by repeated washing with 2 ml of RPMI-1640 medium (Sigma, St Louis, MO) and adjusted at a concentration of 1×10^6 cells/sample. These cells were double-stained with phycoerythrin-labelled anti-IgM and FITC-labelled anti-Mac-1 (Pharmingen, San Diego, CA). Controls cells were stained with isotype controls. The analysis was performed in a FACSCalibur (Becton Dickinson, Mountain View, CA).

Adherent peritoneal cells

Peritoneal cells were collected from the abdominal cavity of mice by repeated lavage with 2 ml of RPMI-1640 medium (Sigma). Cell viability was evaluated using the Trypan blue dye exclusion method. Cells (2×10^5 cells/ml) were dispensed over round glass coverslips (13 mm) in 24-well flat-bottomed microtest plates (Costar) and the cultures incubated at 37° in 5% CO_2 for 60 min. After incubation, the culture supernatants were aspirated and the non-adherent cells removed. Adherent monolayers were rinsed with RPMI-1640. Subsequently, 1 ml of R10 medium (RPMI-1640 containing 10% of heat-inactivated fetal bovine serum) was added to the cultures. The cultures were maintained at 37° in 5% CO_2 for 3 days.

B-1 cell cultures

B-1 cells were cultivated as described by Almeida *et al*.[5] Peritoneal cells from BALB/c mice were obtained as described above. Cells were incubated at 37° in an atmosphere of 5% CO_2 for 7 days. During this period, the culture medium was not changed and B-1 cells grew in these cultures as free-floating cells.[5] The phenotype of these cells was analysed by FACS as described above.

Phagocytosis of zymosan particles

The adherent peritoneal cells were cultured as described previously. After 72 hr, these cells were challenged with 10 μl of a suspension (2 mg/ml) of *Saccharomyces cerevisae* zymosan (Sigma) in phosphate buffered saline (PBS) and incubated at 37° for 30 min. The cultures were then washed to remove the particles not internalized and incubated for 30 min more for the uptake of cell membrane-bound particles. The cultures were washed again and the cells fixed with 0·125% glutaraldehyde (Sigma). The phagocytic index was determined by analysis using phase contrast microscopy. An average of 200 macrophages was counted to determine the phagocytic indexes, calculated as the percentage of phagocytic cells multiplied by the mean number of internalized particles.

IL-10 determination in culture supernatant

Ninety-six-well plates were coated overnight with anti-IL-10 monoclonal antibody produced by the 2A5 hybridoma (Federal University of Rio de Janeiro/UFRJ, Brazil). Plates were washed and blocked with PBS–bovine serum albumin (BSA) 1%. Dilutions of culture supernatants were incubated overnight at 4°, and after washing the wells were incubated with biotin-conjugated anti-IL-10 monoclonal antibodies (mAb) (Pharmingen). After 2 hr incubation, plates were washed; horseradish peroxidase (HRP)–streptavidin conjugate was then added and the plates incubated for an additional hour. After this, the plates were washed and OPD (O-phenylenediamine; Sigma) substrate was added. After developing, the optical density (OD) was determined at 492 nm. The amount of cytokine in each sample was estimated from a standard curve.

NO release in the culture medium

Supernatant from adherent peritoneal cell cultures, stimulated or not with zymozan, were harvested and transferred to 96-well flat-bottomed microtitre plates (Costar). A serial dilution of $NaNO_2$ solution (0·1–6 nmol) was used as

standard curve. Griess reagent solution (1% sulphanilamide, 0·1% naphthylethylenediamine dihydrocloride separately in 2·5% phosphoric acid) was added to the wells containing sample aliquots and $NaNO_2$ dilutions. Thereafter, the plates were read in a microtitre plate reader (MR 4000, Dynatech Chantilly, France) with absorbency at 550 nm. The amount of NO_2 in the culture samples was extrapolated from a standard curve made with different concentrations of $NaNO_2$.

Hydrogen peroxide (H_2O_2) secretion

Peritoneal cells were collected from the peritoneal cavities of mice by repeated lavage with 2 ml of RPMI-1640 medium (Sigma). Cells were adjusted to 2×10^6 cells/ml in a phenol red solution (PBS, 5 mM dextrose, 50 μg of horseradish type II, 0·28 mM phenol red). One hundred μl of the peritoneal cell suspension was dispensed in 96-well flat-bottomed microtitre plates (Costar). Some wells received an additional 2 μl of zymosan suspension (2 ng/ml). The plates were incubated at 37° for 1 hr and the reaction stopped by addition of 10 μl of 1 N NaOH solution.

A standard curve for the determination of H_2O_2 concentration in the experimental tests was constructed using H_2O_2 solutions varying from 50 to 200 nmol of hydrogen peroxide. Absorbance was measured at 620 nm in a microtitre plate reader (Mr 4000, Dynatech). Results were expressed as nanomoles of H_2O_2 per 2×10^6 cells.

Statistics

Statistical comparisons were made by analysis of variance (ANOVA) and by Tukey–Kramer test. All values are reported as the mean ± standard error deviation of the mean with significance assumed in the range of $P < 0{\cdot}05$.

RESULTS

FACS analysis confirms that *Xid* mice are deprived of typical radiosensitive B-1 cells

It has been demonstrated that Xid mice are deficient in B-1 cells;[6] moreover, that these cells are depleted when BALB/c mice are irradiated.[5] Conversely, it is well established that macrophages are radioresistant.[14] In order to confirm these observations further, FACS analysis of peritoneal cells from these mice demonstrates that BALB/c mice have a cell subpopulation that expresses both IgM and Mac-1 (10·13%). Further, when wild-type mice were irradiated (700 rad) the percentage of $IgM^+/Mac\text{-}1^+$ cells decreased to 1% of the total number of peritoneal cells. These data confirm that B-1 cells are radiosensitive.[5] In addition, the persistence of $Mac\text{-}1^+/IgM^-$ cells in the peritoneal cavity of these animals after irradiation confirms the radioresistance of macrophages.[15] Moreover, the data shown here endorse that Xid mice do not have B-1 cells in their peritoneal cavity (Fig. 1a).

When adherent peritoneal cells were cultivated for 7 days, a large number of free-floating small cells were observed in the culture medium of cells obtained from BALB/c mice, as described.[5] Nevertheless, they were neither observed in cultures of peritoneal cells from Balb/Xid nor in cell cultures from irradiated BALB/c mice (Fig. 1b).

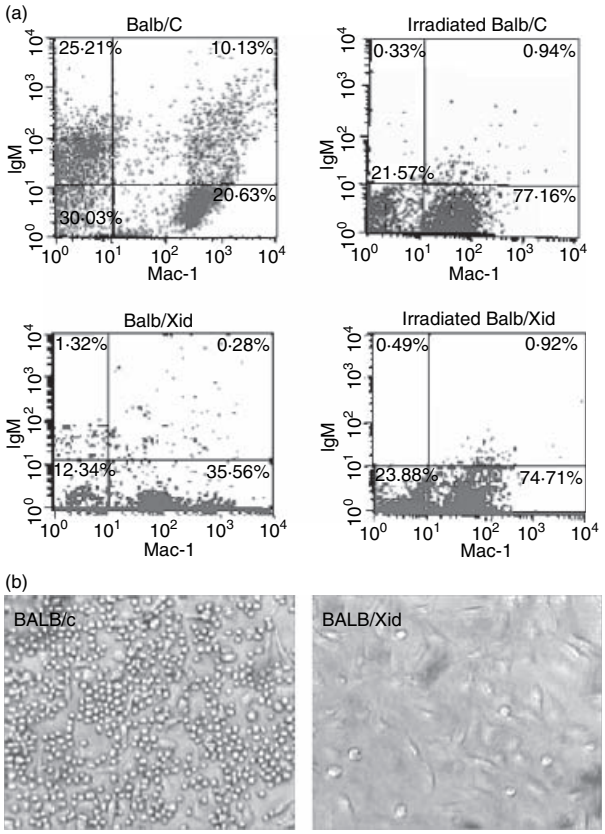

Figure 1. (a) Analysis of peritoneal cell phenotype from BALB/C and BALB/Xid mice, submitted or not to irradiation. Mice were irradiated (700 rad) in a Nordion Gammacell, and 2 days later cells from their peritoneal cavities were collected. These cells were double-stained for IgM (PE antimouse IgM) and Mac-1 (FITC antimouse CD11b) and analysed by flow cytometry. Control cells were stained with isotype controls. (b) Photomicrography of adherent peritoneal cell cultures from BALB/c and irradiated BALB/c mice. Note the absence of small free-floating cells (B-1 cells) in culture of irradiated BALB/c mice. Phase contrast microscopy 40×. Results are representative of three separate experiments.

Macrophages from BALB/Xid or irradiated wild-type mice have higher phagocytic indexes

In order to obtain indirect evidence that B-1 cells might influence macrophage functions, adherent peritoneal cells from BALB/c or BALB/Xid mice, submitted or not to irradiation, were cultivated for 3 days and then incubated with zymosan particles. Macrophages from BALB/Xid mice presented higher phagocytic indexes when compared with phagocytic indexes obtained with cells from BALB/c mice. A significant increase in the phagocytic ability of macrophages from BALB/c mice was observed when these mice were irradiated previously. However, irradiation did not influence phagocytic indexes from adherent peritoneal

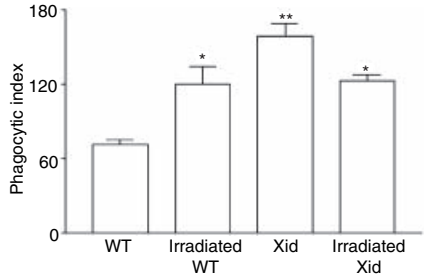

Figure 2. Peritoneal cells from both BALB/c (wild-type) and BALB/Xid (Xid) mice, submitted or not to irradiation were cultivated for 3 days. After this, cells were incubated with zymosan particles. The phagocytic index was evaluated by analysis in phase contrast microscopy. $*P < 0{\cdot}05$ and $**P < 0{\cdot}01$ when values are compared with wild-type group. All statistical analyses performed by comparison with the Xid-irradiated group are not significant ($P > 0{\cdot}05$). Results are expressed as the mean ± SD of four to six samples from a representative experiment performed at least three times.

cells from Xid mice (Fig. 2). These data show indirect evidence that B-1 cells could modulate the phagocytic ability of macrophages *in vitro*.

Macrophages from both *Xid* and irradiated mice release higher amounts of NO and H_2O_2 metabolites

Results demonstrate that macrophages from Xid or irradiated wild-type mice (data not shown) release higher amounts of intermediary reactives of both nitrogen and oxygen. Nitric oxide (NO) release was enhanced greatly when macrophages from Xid mice were stimulated by zymosan. Conversely, zymosan particles were barely able to stimulate NO release by macrophages from the wild-type mice (Fig. 3a).

Figure 3(b) shows that the basal levels of H_2O_2 liberation by cells from Xid mice are higher when compared to cells obtained from wild-type mice. Hydrogen peroxide (H_2O_2) release was increased significantly when macrophages from both wild-type and Xid mice were stimulated with zymosan particles. However, release of H_2O_2 by macrophages from Xid mice was increased when compared to the amount of metabolite released by cells from the wild-type mice. Again, these data suggest that B-1 cells could be down-regulating macrophage effector functions.

Free-floating B-1 cells down-regulate *Xid* peritoneal macrophage activity

The data described above suggest that free-floating B-1 cells in the supernatant of adherent peritoneal cell cultures might be down-regulating macrophage activity. In order to investigate this hypothesis, free-floating B-1 cells were added to macrophages from Xid mice.

B-1 cells were obtained as described by Almeida *et al.*[5] Adherent peritoneal cells from BALB/C mice were cultivated and after 5 days, free-floating cells were harvested and prepared for FACS analysis. Results showed that 85%

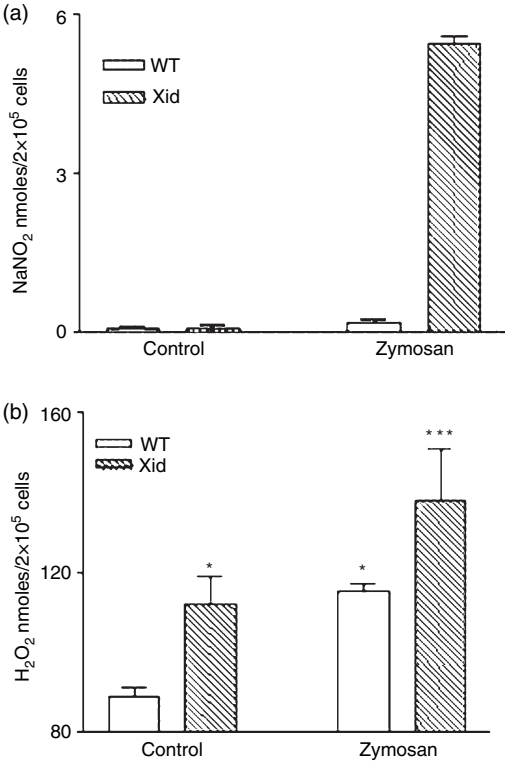

Figure 3. NO (a) and H_2O_2 (b) release by peritoneal macrophages from wild-type and Xid mice, stimulated or not by zymosan particles. $***P < 0{\cdot}001$ when all values are compared with Xid zymosan-treated cells. $*P < 0{\cdot}05$ when values from zymosan wild-type cells and Xid control groups are compared with the wild-type control group. Statistical analyses performed by comparison with other groups do not show statistical significance. Data are mean ± SD of three independent determinations.

of the total cell population of these samples was concomitantly IgM^+ and Mac-1^+ (Fig. 4a).

The addition of free-floating B-1 cells to Xid-derived cultures down-regulated macrophage phagocytic functions (Fig. 4b). NO release by Xid macrophages was also decreased (data not shown), thus suggesting that undetermined factor/s produced by B-1 cells could be responsible for the observed phenomenon.

In order to clarify if contact between these two cell populations was necessary to induce the above-described phenomenon, B-1 cells were cocultivated with adherent peritoneal cells obtained from Xid and wild-type mice in transwell chambers.

Peritoneal cells from wild-type and Xid mice were allowed to adhere to glass coverslips placed on the bottom of 24-well plastic transwell plates. After 3 days of culture, B-1 cells or R-10 medium alone were added to the upper compartment. These cocultures were maintained for 24 hr, when phagocytic tests were performed with macrophages from the bottom of the wells. As shown in Fig. 4(c), phagocytosis by cells from Xid mice at the bottom of the wells was decreased when B-1 cells were added to the upper compartment. Thus, it is concluded that B-1 cell-secreted

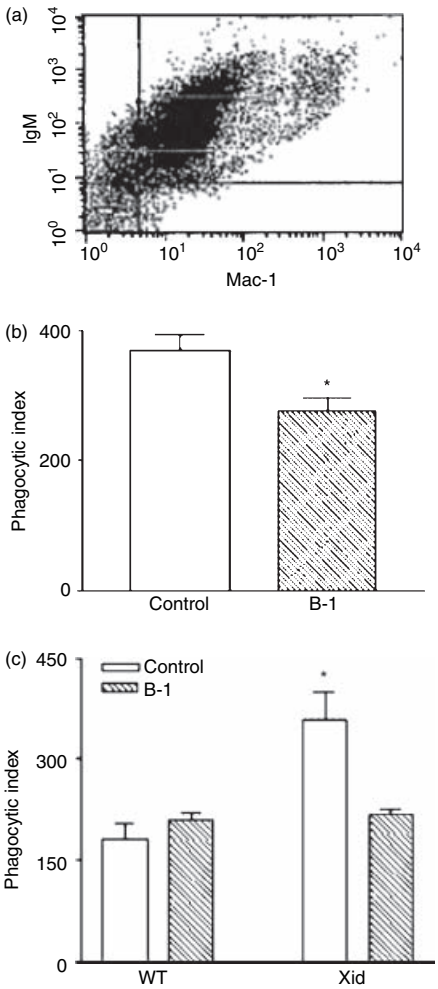

Figure 4. (a) Analysis of IgM and Mac-1 expression by free-floating cells obtained from wild-type adherent peritoneal cell cultures. Control cells were stained with isotype control. (b) Adherent peritoneal cells from Xid mice were cultivated for 3 days when B-1 cells (1×10^4 cells/well) or only culture medium was added. These cocultures were maintained for 24 hr and phagocytic tests were then performed. (c) Adherent peritoneal cells from wild-type and Xid mice were cultivated on the bottom of the transwell chambers. After 3 days, suspension of B-1-enriched cells was added to the upper compartment and 24 hr later phagocytic tests were performed with macrophages on the bottom. $*P < 0{\cdot}05$ when this value are compared with other groups. Results are expressed as the mean ± SD of five samples from a representative experiment performed at least three times.

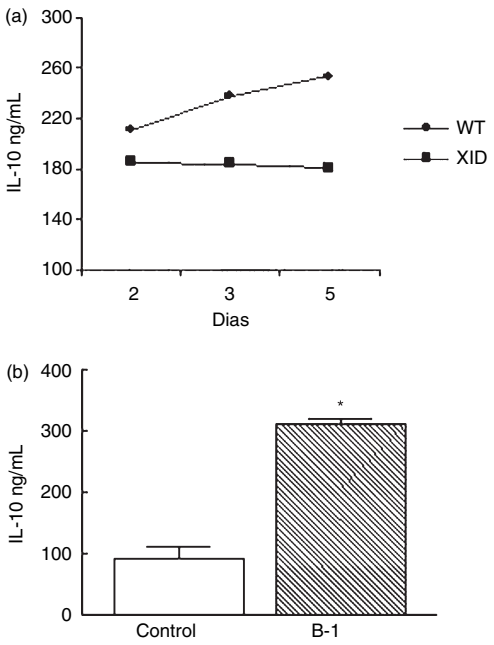

Figure 5. (a, b) Measurement of IL-10 by ELISA in the supernatant of culture of adherent peritoneal cells obtained from both Balb/C and Balb Xid mice on different days of culture (a) and in the supernatant of adherent peritoneal cells from Xid mice cultivated with B-1 cells for 3 days (b). $*P < 0{\cdot}01$ when groups are compared. Data are mean ± SD of three independent determinations.

factor/s induce a down-regulation of phagocytosis by macrophages independently of physical interaction.

These effects on macrophage functions might be due to 'contaminant' cells and not to B-1 cells obtained from the peritoneal cell adherent cultures. Experiments in which free-floating cells from peritoneal cell cultures from wild-type mice were transferred to Xid mice rejects this hypothesis. In these conditions the only different cell type transferred to Xid mice was B-1 cells.

B-1 cells release IL-10 in the culture medium

B-1 cells, as well as conventional B cells, produce IL-10.[9] Nevertheless, the influence of B-1-derived IL-10 on macrophage functions has not been investigated. Results show that the amount of IL-10 in the supernatant of adherent peritoneal cell cultures from wild-type mice increases with time. Conversely, although adherent peritoneal cells from Xid mice do secrete IL-10, an increase in the amount of this cytokine with time could not be observed (Fig. 5a). However, when adherent peritoneal cells from Xid mice were cultivated with wild-type derived B-1 cells (as described in Fig. 4), a threefold increase in IL-10 concentration in culture medium was detected (Fig. 5b). These data indicate strongly that the increase in IL-10 secretion by B-1 cells is the factor responsible for the observed down-regulation of macrophages.

B-1 cells from KO IL-10 mice do not modulate macrophage functions

In an attempt to demonstrate this assumption, B-1 cells from KO IL-10 mice were cocultivated with macrophages from wild-type irradiated mice, as described previously. After 3 days of cocultures phagocytic tests were performed. It was confirmed by enzyme-linked immunosorbent assay (ELISA) that macrophages cocultivated with IL-10 KO-derived B-1 cells did not have measurable levels of IL-10; otherwise, macrophages cocultivated with wild-type

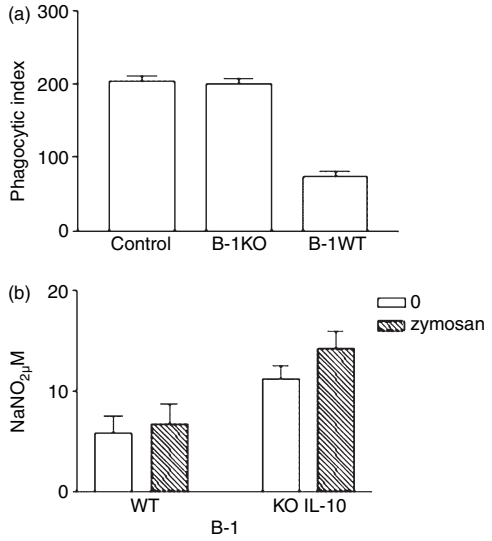

Figure 6. (a) Phagocytosis by adherent peritoneal cells from BALB/c-irradiated mice cocultivated with culture medium (control), wild-type B-1 cells or IL-10 KO B-1 cells performed as described before in Figure 4. (b) NO release by adherent peritoneal cells from wild-type irradiated mice cocultivated with B-1 cells from wild-type or IL-10 KO mice stimulated or not with zymosan. Results are expressed as the mean ± SD of three separate experiments.

B-1 cells have normal amounts of IL-10, as verified previously (data not shown). Results show that IL-10 KO-derived B-1 cells were not able to inhibit macrophage functions as did B-1 cells from wild-type mice (Fig. 6a). Furthermore, B-1 cells from KO IL-10 mice did not inhibit NO release by macrophages as observed previously, when wild-type-derived B-1 cells were used (Fig. 6b).

These data confirm the involvement of IL-10 in the phenomenon described here, allowing us to postulate that IL-10 secreted by B-1 cells is the main suppressor factor of macrophage functions in these cultures.

DISCUSSION

The role of B-1 cells on the kinetics and fate of the inflammatory response has been poorly investigated. However, there is evidence that they migrate to a non-specific inflammatory focus.[5,16]

A large number of reports, including the results described in this study, demonstrate that Xid mice do not have B-1 cells in the peritoneal and pleural cavities.[6] Recently, Riggs *et al.*[17] questioned these observations with data that support the existence of B-1 cells in the peritoneal cavity of mice, a controversy to be clarified. Nevertheless, it has been demonstrated that Xid mice are more resistant to some infections.[7,8,18] These data suggest that B-1 cells induce down-regulation of effector cells, such as macrophages, in the course of infectious diseases. Results demonstrated in this study support this hypothesis indirectly.

It was shown clearly that adherent peritoneal cells from Xid mice have a higher phagocytic ability when compared with adherent peritoneal cells collected from wild-type mice. Nevertheless, when wild-type mice were irradiated previously the phagocytic indexes of peritoneal adherent cells collected from these animals increased. The mice submitted to lethal irradiation had decreased the B-1 cell population and increased the number of Mac-1^+ cells in the peritoneal cavity. Evidence that irradiation *per se* does not increase the phagocytic ability of macrophages came from results obtained with adherent peritoneal cells from normal and irradiated Xid mice. These observations led us to hypothesize that B-1 cells which are radiosensitive[5] might be regulating macrophage functions.

Supporting this hypothesis, the addition to B-1 cells to adherent peritoneal cells from Xid mice decreased their phagocytic indexes via mannose/β-glucan receptors.[19] The same effect was observed when opsonized sheep red blood cells were tested (data not shown), thus indicating that both mannose/β-glucan and Fc receptors were down-regulated by the presence of B-1 cells. This observation posed two distinct interpretations: either B-1 cells secret a soluble down-regulatory factor of macrophage functions, or the physical contact between B-1 cells and macrophages could be responsible for the observed phenomenon. Experiments using transwell apparatus support the interpretation that B-1 cells secret in culture a factor or factors that down-regulate macrophage functions.

The down-regulatory effect of B-1 cells on macrophage functions is not limited to its phagocytic ability. Results show that the release of intermediary reactives of nitrogen and oxygen by macrophages is also impaired in the presence of B-1 cells.

Among the constellation of factors that might be exerting this down-regulatory effect on macrophages, IL-10 was considered to be one of the possible candidates, as it has been demonstrated that IL-10 suppresses the development of cell-mediated immunity by inhibiting macrophage functions.[20–22] Further, this cytokine inhibits the generation of reactive oxygen and nitrogen intermediate[11] and macrophage activation.[13] It is also known that B-1 cells, B-1a and B-1b, both produce and utilize IL-10 as an autocrine growth factor.[9]

Corroborating this interpretation, we have shown that adherent peritoneal cells from wild-type mice produce higher amounts of IL-10 when compared to cultures of adherent cells obtained from Xid mice which lack B-1 cells in their peritoneal cavity. The addition of B-1 cells to cultures of adherent peritoneal cells from Xid mice increased the amount of IL-10 secreted in the culture medium, further supporting the hypothesis that the B-1 cell down-regulatory effect on macrophages might be mediated by IL-10.

This interpretation was demonstrated clearly when IL-10 KO-derived B-1 cells was not able to down-regulate macrophage functions. Macrophages cocultivated with IL-10 KO B-1 cells did not decrease phagocytic index and NO release, as observed when B-1 cells from wild-type mice were added to macrophage cultures.

Taken together, these data demonstrate clearly that B-1 cells down-regulate macrophage functions *in vitro*.

Considering that these cells migrate to an inflammatory milieu, where they become a mononuclear phagocyte, it is reasonable to speculate that B-1 cells might be exerting a down-regulatory effect on inflammatory macrophages thus, on one hand, impairing tissue damage and on the other hand, facilitating the persistence of parasites in the host tissues.

ACKNOWLEDGMENT

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REFERENCES

1 Herzenberg LA, Kantor AB. B-cell lineages exist in the mouse. Immunol Today 1993; **14:**79–83; discussion 88–90.
2 Herzenberg LA, Stall AM, Lalor PA, Sidman C, Moore WA, Parks DR. The Ly-1 B cell lineage. Immunol Rev 1986; **93:**81–102.
3 Hayakawa K, Hardy RR, Parks DR, Herzenberg LA. The "Ly-1 B" cell subpopulation in normal immunodefective, and autoimmune mice. J Exp Med 1983; **157:**202–18.
4 Kantor AB, Herzenberg LA. Origin of murine B cell lineages. Annu Rev Immunol 1993; **11:**501–38.
5 Almeida SR, Aroeira LS, Frymuller E, Dias MA, Bogsan CS, Lopes JD, Mariano M. Mouse B-1 cell-derived mononuclear phagocyte, a novel cellular component of acute non-specific inflammatory exudate. Int Immunol 2001; **13:**1193–201.
6 Narendran A, Ramsden D, Cumano A, Tanaka T, Wu GE, Paige CJ. B cell developmental defects in X-linked immunodeficiency. Int Immunol 1993; **5:**139–44.
7 Minoprio P, el Cheikh MC, Murphy E, Hontebeyrie-Joskowicz M, Coffman R, Coutinho A, O'Garra A. Xid-associated resistance to experimental Chagas' disease is IFN-gamma dependent. J Immunol 1993; **151:**4200–8.
8 Paciorkowski N, Porte P, Shultz LD, Rajan TV. B1 B lymphocytes play a critical role in host protection against lymphatic filarial parasites. J Exp Med 2000; **191:**731–6.
9 O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. Eur J Immunol 1992; **22:**711–7.
10 Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol 1993; **11:**165–90.
11 Bogdan C, Vodovotz Y, Nathan C. Macrophage deactivation by interleukin 10. J Exp Med 1991; **174:**1549–55.
12 Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. J Immunol 1988; **141:**2407–12.
13 Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991; **147:**3815–22.
14 Gillette RW, Lance EM. Kinetic studies of macrophages. IV. Effect of irradiation. J Reticuloendothel Soc 1973; **14:**18–25.
15 Coutier S, Bezdetnaya L, Marchal S, Melnikova V, Belitchenko I, Merlin JL, Guillemin F. Foscan (mTHPC) photosensitized macrophage activation: enhancement of phagocytosis, nitric oxide release and tumour necrosis factor-alpha-mediated cytolytic activity. Br J Cancer 1999; **81:**37–42.
16 Askenase PW, Kawikova I, Paliwal V, Akahira-Azuma M, Gerard C, Hugli T, Tsuji R. A new paradigm of T cell allergy: requirement for the B-1 cell subset. Int Arch Allergy Immunol 1999; **118:**145–9.
17 Riggs J, Howell K, Matechin B, Matlack R, Pennello A, Chiasson R. X-chromosome-linked immune-deficient mice have B-1b cells. Immunology 2003; **108:**440–51.
18 Hoerauf A, Solbach W, Lohoff M, Rollinghoff M. The Xid defect determines an improved clinical course of murine leishmaniasis in susceptible mice. Int Immunol 1994; **6:**1117–24.
19 Underhill DM, Ozinsky A. Phagocytosis of microbes: complexity in action. Annu Rev Immunol 2002; **20:**825–52.
20 Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by $B220^+$ cells from schistosome-infected mice: a mechanism for regulation of $CD4^+$ T-cell subsets. Proc Natl Acad Sci USA 1994; **91:**18–22.
21 Tripp CS, Wolf SF, Unanue ER. Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. Proc Natl Acad Sci USA 1993; **90:**3725–9.
22 D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J Exp Med 1993; **178:**1041–8.