Regulatory B Cells Shape the Development of Th2 Immune Responses in BALB/c Mice Infected with *Leishmania major* Through IL-10 Production

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Recent evidence indicates that B cells are required for susceptibility to infection with *Leishmania major* in BALB/c mice. In this study, we analyzed the role of the IL-10 produced by B cells in this process. We showed that B cells purified from the spleen of BALB/c mice produced IL-10 in response to stimulation with *L. major* in vitro. In vivo, early IL-10 mRNA expression is detected after *L. major* infection in B cells from draining lymph nodes of susceptible BALB/c, but not of resistant C57BL/6 mice. Although adoptive transfer of naive wild-type B cells prior to infection in B cell-deficient BALB/c mice restored Th2 cell development and susceptibility to infection with *L. major* of these otherwise resistant mice, adoptive transfer of IL-10−/− B cells mice did not. B cells stimulated by *L. major*, following in vitro or in vivo encounter, express the CD1d and CD5 molecules and the IL-10 produced by these cells downregulate IL-12 production by *L. major*-stimulated dendritic cells. These observations indicate that IL-10 secreting B cells are phenotypically and functionally regulatory B cells. Altogether these results demonstrate that the IL-10 produced by regulatory CD1d+ CD5+ B cells in response to *L. major* is critical for Th2 cell development in BALB/c mice. The Journal of Immunology, 2010, 184: 000–000.

B cells are also able to produce regulatory cytokines such as IL-10 in vitro (5–7). The in vivo role of IL-10-producing B cells was first demonstrated in a murine model of experimental autoimmune encephalomyelitis (EAE). In this model, the exacerbated encephalomyelitis that develops after immunization with myelin oligodendrocyte glycoprotein peptide in B cell-deficient mice was demonstrated to be the consequence of a deficiency of IL-10-producing B cells (8). Indeed, in the absence of IL-10 production by B cells, the proinflammatory type 1 immune response persisted, and mice did not recover (8). The involvement of the IL-10–producing B cells named regulatory B (Breg) cells was then extended to immunopathology developing in other autoimmune diseases such as collagen induced arthritis, ulcerative colitis, and lupus (9–11).

Recently, IL-10 production by B cells was also described during parasitic diseases. B cells from mice infected with *Schistosoma mansoni* proliferate, secrete IL-10, and promote the development of *Leishmania* immune response (12). Purified B cells from BALB/c mice infected with *Leishmania major*, *Brugia malayi*, or *Brugia pahangi* produced IL-10 in response to restimulation with soluble *Leishmania* or microfilarial extract, respectively (5, 13). In addition, it was recently described that stimulation of naive splenic BALB/c B cells with *Leishmania infantum* tryparedoxin lead to IL-10 production (14). Altogether, these observations demonstrate that B cells could produce IL-10 in response to parasite Ags. However, the exact role of the IL-10 producing B cells in vivo in parasitic disease is not yet established.

Studies in vivo, using the murine model of infection with *L. major*, have established that resistance and susceptibility to infection with this protozoan parasite are correlated with the activation of parasite-reactive CD4+ Th1 or Th2 cells, respectively (15). IL-4 has been shown for a long time to play a predominant role in directing the functional differentiation of *Leishmania* reactive CD4+ Th2 T cells observed in susceptible BALB/c mice postinfection with *L. major* (16). However, the use of IL-4Rα−/− BALB/c mice has generated controversial results. Indeed, IL-4Rα−/− BALB/c mice infected with *L. major* either contained
infection (17) or still developed progressive disease (18). These observations suggest that another cytokine than IL-4 might be involved in susceptibility to infection with L. major. Results showing that IL-10−/− and IL-4Δα−/− BALB/c mice control parasite replication demonstrate that IL-10 signaling contribute to susceptibility to L. major infection (19).

We have recently demonstrated that B cells are required for susceptibility and Th2 cell development in BALB/c mice infected with L. major LV39 (20). We (20) and others (21) have also established that the ability of B cells to direct the immune response in BALB/c mice toward a Th2 phenotype was dependent upon their capacity to present Ag to T cells rather than upon their production of specific IgG Abs.

The aim of the current study was to analyze the role of cytokine secreted by B cells, particularly IL-10, in the polarization of the Th2 cell response and the susceptibility of BALB/c mice to infection with L. major LV39. We clearly demonstrated that IL-10 produced by B cell is necessary for susceptibility to infection with L. major LV39 in BALB/c mice and for the Th2 cell development. The effect of IL-10 producing B cells was shown, at least in vitro, to stem IL-12 production by DCs.

Materials and Methods

Mice

µMT mice on the C57BL/6 background were obtained from Kitamura et al. (22). These mice were backcrossed 10 times to the BALB/c background. Flow cytometry analysis was used to confirm the absence of CD19+ cells in the peripheral blood of these mice. IL-10−/− BALB/c mice were kindly provided by Dr. J. P. Farewell (University of Pennsylvania, Philadelphia, PA). Female BALB/c and C57BL/6 mice were purchased from Harlan (Horst, The Netherlands). Mice were bred and maintained in the animal facilities of the Swiss Institute for Experimental Cancer Research under pathogen-free conditions (Epalinges, Switzerland). The maintenance and care of mice complied with the guidelines of the University of Lausanne Ethic Committee.

Parasites and infection

L. major LV39 (MRHO/SU/59/P), isolated from a gerbil reservoir in southern Russia, were maintained in vivo and grown in vitro as previously described (23). For infection, mice were injected in hind footpads with 3 × 10^6 stationary phase L. major promastigotes in a volume of 50 μl of DMEM. Size of footpad lesions was measured with a Vernier caliper and compared with the thickness of the uninfected footpad. Footpad tissues were used to create limiting dilutions for quantification of viable parasite burdens as previously described (23).

MACS and adoptive transfer of B cells to BALB/c µMT mice

B cells were purified from spleen cells from either naive BALB/c or IL-10−/− BALB/c mice using Magnetic Activated Cell Sorting (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany). Briefly, total spleen cells were incubated with a mixture of biotin-conjugated Abs and anti-Biotin microbeads against CD43 (Ly-48), CD4 (L3T4), and Ter-119 to remove T cells, NK cells, DCs, macrophages, granulocytes, and erythrocyte cells. After immobilization of all these cells with a magnet, untouched CD19+ naive cells, which was defined as the reference.

Lymphocyte stimulation and detection of cytokines in supernatants

Total LN cells. Popliteal LN cells (5 × 10^6) were stimulated with UV-irradiated L. major promastigotes (1 × 10^6) in a final volume of 1 ml. Cells were cultured in DMEM supplemented with 5% heat inactivated FCS, 2 mM L-glutamine, 5 × 10^−4 M 2-μME, and 10 mM Heps in an atmosphere of 7% CO2 at 37°C. All media and FCS used in this study were certified endotoxin free. Culture supernatants were collected after 72 h and stored at −20°C until use.

B cells. B cells purified by MACS (2 × 10^6 cells) were stimulated in vitro with complete DMEM medium alone, L. major live promastigotes (1–5 parasites/cell), LPS (1 μg/ml, Escherichia coli, serotype 055:B5; Sigma-Aldrich, Epalinges, Switzerland), CpG (1 μg/ml, ODN1826; InvivoGen, Toulouse, France). Culture supernatants were collected after 24 h and stored at −20°C until use.

ELISA. IFN-γ was measured in supernatants by ELISA as described (20). Mouse recombinant IFN-γ (supernatant of L1210 cells transfected with the murine IFN-γ, a gift of Y. Watanabe, Kyoto University, Kyoto, Japan) was used as standard. IL-10 and IL-4 were measured by ELISA using commercial kits (BD Biosciences, Allschwil, Switzerland). The limits of detection of these assays were 10 IU/ml for IFN-γ and 20 pg/ml for IL-4 and IL-10. An ELISA kit with a sensitivity of 15 pg/ml was used for the detection of IL-12p70 (bEbioscience).

FACS analysis

Single-cell suspensions were first incubated 10 min with an anti-FcγRII/III mAb (Fcblock, 2.4G2), followed by a 20 min exposure to cell surface staining performed with the following Abs: CD19 (1D3), CD21 (7G6), CD23 (B3B4), IgM (DS-1), and IgD (AMS9.1). mAbs were obtained from BD Biosciences, at 73.73 antigen was quenched by eBioscience. PE-cychrome5-streptavidin was purchased from BD Biosciences. Cells were analyzed in PBS containing 3% FCS using a FACScalibur flow cytometer (BD Biosciences) and FlowJo software (TreeStar, Ashland, OR).

Flow cytometry analysis of IL-10–producing B cells

Splenic or LN B cells were purified by MACS and were cultured in medium alone, or with either L. major or with CpG, during 24 h. IL-10–producing cells were isolated using the IL-10 secretion assay in accordance to the manufacturer’s instructions (Miltenyi Biotec). Briefly, a rat anti-IL-10 Ab conjugated to cell surface marker specific Ab (IL-10 catch reagent) was attached at the surface of B cells. Cells were then incubated 45 min at 37°C and IL-10 secreted bound to IL-10 catch reagent on the surface. Thereafter, enriched IL-10–secreting B cells were stained for specific markers (CD1d, CD5, IgM, IgD, CD21, CD23) to phenotype further IL-10+ B cells.

RNA extraction, cDNA preparation, and real-time RT-PCR

Total RNA was isolated from total LN cells or purified cells using RNeasy Mini Kit (Qiagen, Hombrechtikon, Switzerland) according to the manufacturer’s instructions. cDNA synthesis was carried out following standard procedures using dNTPs (Roche, Basel, Switzerland) and Superscript II reverse transcriptase (Invitrogen, Basel, Switzerland) according to the manufacturer’s instruction. PCR amplification was prepared using LightCycler Faststart DNA Master SYBR Green I kit (Roche Diagnostics AG, Rotkreuz, Switzerland) and quantitative PCR amplification was performed with the LightCycler 1.5 system (Roche) according to the manufacturer’s instructions. The primers for the real-time PCR were the following: HPRT Fw 5′-GTTGGATATGCCCTTGAC-3′; Rev 5′-AGGACTAGAACACCTGCT-3′; Rev 5′-AGGACTAGAACACCTGCT-3′; IL-10 Fw 5′-ACCTGCTCCA CGTCCTTGTGTC-3′; and IL-10 Rev 5′-GGTTGGCAAGGCTTATCGGA-3′. The results were analyzed using the comparative threshold cycle method (ΔΔCt) for relative quantification of gene expression normalized to the housekeeping gene HPRT. In some experiments, results were expressed as the fold change of the IL-10 mRNA in cells from infected mice compared with naive cells, which was defined as the reference.

Dendritic cell generation and coculture

Bone marrow cells were flushed from long bones with RPMI. Erythrocytes were lysed with RBC lysis buffer (150 mM NH4Cl, 1.0 mM KHCO3, and 0.1 mMNa2EDTA). DCs were generated by plating of bone marrow progenitors (2 × 10^6/ml) in complete RPMI medium supplemented with 30% conditioned medium from GM-CSF–transduced AO-2 cells as previously described (24). After 3 d, fresh DC medium was added. On day 6, DCs were recovered and stimulated with L. major promastigotes (ratio 5:1) or LPS (1 μg/ml).

In some experiments, DCs were stimulated in a final volume of 400 μl during 24 h with 200 μl medium or L. major promastigotes in presence of 200 μl of B cell supernatants that have been stimulated with medium alone or with L. major.

Results

B cells produce IL-10 after stimulation by L. major in vitro

Cytokine production by B cells purified from spleens of naive BALB/c mice stimulated with L. major promastigotes (from one to five parasites/cell) was analyzed. Although IL-10 was already produced by B cells from naive BALB/c mice in response to
infection is critical for the differentiation of CD4+ T cells toward the draining LN cells determined. Results in Fig. 2 major promastigotes and the kinetics of IL-10 mRNA expression in produced during this critical window of time, susceptible BALB/c Th1 or Th2 phenotype (25, 26). Thus, to determine whether IL-10 was susceptibility to the IL-10 produced by B cells was necessary or not to redirect of the adoptive transfer of naive B cells from IL-10 into otherwise resistant BALB/c We have previously reported that adoptive transfer of naive B cells L. major modify the resistance of these mice to infection with L. major. It has been clearly established that, in the murine model of infection with L. major, the cytokine environment during the early phases of infection is critical for the differentiation of CD4+ T cells toward the Th1 or Th2 phenotype (25, 26). Thus, to determine whether IL-10 was produced during this critical window of time, susceptible BALB/c and resistant C57BL/6 mice were infected in the footpads with L. major promastigotes and the kinetics of IL-10 mRNA expression in draining LN cells determined. Results in Fig. 2A show that IL-10 mRNA expression was detected in both susceptible BALB/c and resistant C57BL/6 mice during the first 3 d of infection with a peak on day 1 that rapidly returned to the baseline levels at day 3.

To determine whether B cells are responsible for the IL-10 mRNA burst detected postinfection with L. major, B cells were purified from draining LN cells of both BALB/c and C57BL/6 mice and analyzed for IL-10 mRNA expression. Results clearly showed an increase of IL-10 mRNA transcripts 1 d postinfection with L. major exclusively in CD19- cells in C57BL/6 mice but in both CD19+ and CD19- cells in BALB/c mice (Fig. 2B). These data suggest that B cells are a source of IL-10 in BALB/c mice infected with L. major LV39. However, it is necessary to note that only living parasites induce this phenotype, because when BALB/c mice were inoculated with SLA, a peak of IL-10 mRNA transcripts was observed in draining LN cells but mainly in CD19- cells (data not shown).

Transfer of IL-10-/- B cells into μMT BALB/c mice did not modify the resistance of these mice to infection with L. major We have previously reported that adoptive transfer of naive B cells into otherwise resistant BALB/c μMT mice restored susceptibility to infection with L. major (20). In an attempt to determine whether the IL-10 produced by B cells was necessary or not to redirect susceptibility to L. major in BALB/c mice, we analyzed the effect of the adoptive transfer of naive B cells from IL-10-/- BALB/c mice into BALB/c μMT on the course of infection and the pathway of Th cell differentiation.

As already demonstrated, BALB/c μMT mice infected with L. major LV39 were resistant to infection. Whereas BALB/c μMT mice reconstituted with wild-type (WT) B cells developed progressive lesions similar to those observed in BALB/c mice, BALB/c μMT mice reconstituted with IL-10-/- B cells controlled the development of lesions (Fig. 3A). At the time of parasite burden determination, the percentages of draining LN B cells were: 23.4% ± 3.8% and 26.6% ± 2.3% of lymphocytes in BALB/c μMT reconstituted with WT B cells or IL-10-/- B cells, respectively. Estimation of the number of parasites in lesions clearly showed that parasites growth was controlled in BALB/c μMT mice reconstituted with IL-10-/- B cells but not in BALB/c μMT mice reconstituted with WT B cells (Fig. 3B).

Analysis of cytokines production in supernatants of specifically stimulated draining LN cells at the end of infection (day 48) revealed that whereas BALB/c μMT mice reconstituted with WT B cells

CD19+ cells from BALB/c mice produced IL-10 in vivo during the first days of infection with L. major It has been clearly established that, in the murine model of infection with L. major, the cytokine environment during the early phases of infection is critical for the differentiation of CD4+ T cells toward the Th1 or Th2 phenotype (25, 26). Thus, to determine whether IL-10 was produced during this critical window of time, susceptible BALB/c and resistant C57BL/6 mice were infected in the footpads with L. major promastigotes and the kinetics of IL-10 mRNA expression in draining LN cells determined. Results in Fig. 2A show that IL-10 mRNA expression was detected in both susceptible BALB/c and resistant C57BL/6 mice during the first 3 d of infection with a peak on day 1 that rapidly returned to the baseline levels at day 3.

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mounted a typical Th2 cell response with high levels of IL-4 similar to those observed in infected BALB/c mice, BALB/c μMT mice reconstituted with IL-10−/−B cells developed a Th1 response with high levels of IFN-γ and low levels of IL-4 (Fig. 3C). IFN-γ and IL-4–producing cells were analyzed by intracellular staining. The absolute number of IFN-γ–producing cells among the CD4+ T cells in the draining LN was similar among all the group of mice. In contrast, five times more CD4+ IL-4+ cells were measured in BALB/c μMT mice and μMT mice reconstituted with WT B cells than in μMT mice and μMT mice reconstituted with IL-10−/− B cells (data not shown).

IL-10 production was also analyzed in supernatants of draining LN stimulated with L. major. First and interestingly, cells from BALB/c μMT produced lower IL-10 levels than cells from BALB/c mice. In addition, whereas draining LN cells from BALB/c μMT mice reconstituted with WT B cells produced IL-10 in response to stimulation with L. major in a similar amount compared to cells from BALB/c mice, cells from BALB/c μMT mice reconstituted with IL-10−/− B cells did not (Fig. 3C).

These results indicate that the IL-10 produced by B cells is involved in the induction of a Th2 cell response and in susceptibility to L. major LV39 in BALB/c mice.

IL-10 produced by B cells downregulates IL-12 production by DCs in vitro after L. major stimulation

IL-10 is a potent antiinflammatory cytokine, which was identified as a Th1 inhibitor due to its ability to prevent IL-12 secretion by DCs (27). Thus, we analyzed the effect of the IL-10 produced by B cells stimulated with L. major in response to this parasite.

First, we analyzed IL-12p70 production by bone marrow DCs (BMDCs) derived from WT BALB/c mice in response to L. major stimulation. As shown in Fig. 4A, BMDCs from BALB/c mice did not produce IL-12p70 in response to L. major stimulation. As a control, substantial amount of IL-12p70 (500 pg/ml) was produced by BMDCs cells in response to stimulation by LPS (Fig. 4A). Interestingly, stimulation of BMDCs from BALB/c mice with L. major induced some IL-12 production (Fig. 4A). As IL-10 is described as a cytokine able to prevent IL-12 production by DCs, similar DC stimulations were done with BMDCs from IL-10−/− BALB/c. As shown in Fig. 4B, DCs from IL-10−/− BALB/c mice produced significant amount of IL-12p70 in response to L. major. These results suggest that autocrine IL-10 produced by BMDCs in response to L. major stimulation downregulate IL-12p70 production.

As BMDCs from IL-10−/− BALB/c produced significant amount of IL-12p70 after L. major stimulation, these cells were further used to analyze the suppressive effect of IL-10 secreted by B cells on DC activity.

First, we analyzed the effect of IL-10 produced by WT B cells in vitro in response to L. major on the capacity of BMDCs from IL-10−/− BALB/c mice to produce IL-12p70. Thus, BMDCs from IL-10−/− BALB/c mice were stimulated during 24 h with L. major in the presence of supernatants of either unstimulated or L. major–stimulated B cells. Fig. 4C clearly shows that in contrast to supernatants of unstimulated B cells, supernatants of BALB/c B cells stimulated with L. major inhibited IL-12p70 production of IL-10−/− BMDCs stimulated with L. major. The same results were obtained with supernatants of LPS-stimulated B cells (data not shown).
To confirm that IL-10 present in supernatants of B cells stimulated by L. major was indeed responsible for IL-12p70 downregulation observed above in IL-10−/− BMDCs (Fig. 4C), IL-10−/− BMDCs were stimulated by L. major in the presence of supernatants from IL-10−/− B cells stimulated with L. major. Fig. 4D clearly shows that supernatants of IL-10−/− B cells stimulated with L. major did not inhibit the IL-12p70 produced by IL-10−/− BMDCs stimulated by L. major. Altogether, these results demonstrated that IL-10 produced by B cells in response to L. major downregulate the IL-12p70 produced by BMDCs.

Unfortunately, we were unable to study the regulation by B cells derived IL-10 on IL-12p70 production by DCs in vivo during the early steps of infection, because induction of IL-12p70 in L. major infected mice is restricted to few numbers of DCs during the first days of infection (28).

Characterization of B cell-producing IL-10 in response to L. major

In order to determine the phenotype of B cells secreting IL-10 after L. major stimulation in vitro, we enriched the IL-10−producing B cells with an IL-10 secretion assay kit (Miltenyi Biotec) and analyzed the expression of specific markers of B cells on the IL-10 negative and positive enriched B cell population.

As shown in Fig. 5A, IL-10−producing B cells were mostly CD5+ after stimulation with L. major (76.5%) or with CpG (70.2%). Interestingly, the few IL-10−producing B cells detected
in unstimulated culture were also CD5+ (71.2%). However, whereas 35.9% of IL-10–producing cells were CD5+ and CD1d+ after L. major stimulation, only 16.6% of IL-10–producing B cells in responses to CpG were CD5+ and CD1d+ (Fig. 5A). As a control, without any stimulation, 8.8% of IL-10–producing B cells are CD1d+ and CD5+ (Fig. 5A). In addition, non-IL-10–producing B cells are CD5+ and CD1d+ whatever the stimulation used.

Expression of characteristic markers of B cell subsets such as CD21, CD23, IgD, and IgM were also analyzed in IL-10–producing B cells. A downregulation of CD21 and CD23 expression was observed in B cells stimulated with L. major as compared with unstimulated or CpG stimulated B cells (58.2% versus 92.7% and 91%, respectively, for CD21; 53.2% versus 90.9% and 76.5%, respectively, for CD23). Whereas no difference was observed in IgM expression in B cells whatever the stimulus used (L. major or CpG), a downregulation of IgD was detected in IL-10–producing B cells after L. major and CpG stimulation as compared with unstimulated B cells.

Altogether these results demonstrated that in vitro IL-10–producing B cells after L. major are CD5+, CD1d+, CD21+, and CD23− and that this phenotype is specific to L. major because IL-10–producing B cells after stimulation with CpG are CD5+, CD1d−, CD21high, and CD23− (Fig. 5B). As controls, only 0.91% non-IL-10–producing B cells are CD5+ and CD1d+ 1 d post-infection with L. major.

In conclusion, IL-10 production by B cells after L. major stimulation is confined to a small, discrete population of CD5+ and CD1d+ B cells.

Discussion

Results obtained in this study showed that the IL-10 produced by B cells in response to L. major LV39 plays an important role in the development of susceptibility to the infection. First, we described that purified B cells stimulated in vitro with L. major secrete IL-10. Moreover, IL-10 expression is detected in purified CD19+ cells from draining LN 1 d postinfection with L. major and characterized for their expression of CD5 and CD1d markers. In accordance with the phenotype of IL-10–producing B cells after L. major stimulation in vitro, 14.1% of IL-10–producing B cells 1 d postinfection with L. major are CD5+ and CD1d+ (Fig. 5B). As controls, only 0.91% non-IL-10–producing B cells are CD5+ and CD1d+ 1 d post-infection with L. major.

The absence of IL-10 production by B cells in the first days of infection and the cytokines in addition to IL-4 are responsible for susceptibility to infection with L. major. Indeed, whereas otherwise BALB/c µMT mice reconstituted with WT B cells develop progressive lesions similar to those observed in BALB/c mice, BALB/c µMT mice reconstituted with IL-10−/− B cells contain the development of lesions and develop a Th1 response. The results presented in this paper showing that IL-10 produced by B cells, a potential APC, is necessary for susceptibility to infection with L. major are in agreement with the observations demonstrating that IL-10 produced by APCs is needed for susceptibility to infection with L. major.

The effect of IL-10 on susceptibility of BALB/c mice to L. major infection appears critically dependent on the nature of its cellular source. In this context, it has been demonstrated that Tg C57BL/6 mice expressing IL-10 under the control of the IL-2 promoter, directing the expression of IL-10 in T cells, remained resistant to L. major infection and developed a Th1 response comparable to that of WT C57BL/6 mice (43). In contrast, C57BL/6-resistant mice that express an IL-10 transgene under the control of the MHC II Ea promoter, directing the expression of IL-10 mainly in APCs, were susceptible to L. major (42).

The absence of IL-10 production by B cells in the first days of infection in resistant mice also suggests that in susceptible mice, B cells facilitate lesion development by suppressing the differentiation of protective Th1 effector cells. A recent report showed that CpG-activated neonatal B cells inhibited IL-12 secretion by DC through an IL-10–dependent mechanism (44). Furthermore, in the EAE model, recovery was associated with the suppression of IL-12 production by DCs by IL-10 secreted by B cells (8, 45). In accordance with these results, we demonstrated that L. major-activated
B cells limit the capacity of DCs to produce IL-12. First, supernatants of *L. major* stimulated B cells from BALB/c mice that contained IL-10 inhibit IL-12p70 production of DCs stimulated with *L. major*. Secondly, in contrast to adoptive transfer of WT B cells, adoptive transfer of IL-10−/− B cells did not promote the development of the Th2 immune response in infected mice.

Recently, Breg cells have been identified in experimental models of autoimmunity, cancer, and infections. The regulatory function of these B cells was shown to be mediated by the production of IL-10. Different subsets of Breg cells distinguished by their surface phenotype and their immunoregulatory properties (i.e., down-regulating IL-12 production or costimulatory molecules on APCs...
or even inducing Treg cells) have been described (46). The phenotype of IL-10–producing B cells after L. major stimulation is clearly CD19+ CD5+ CD1d+ CD21low, and CD23low. These B cells share some phenotypic markers with the B cell subsets already described: CD5+ B1 cells, CD1d+ CD23+ IgM+ IgD+ transitional T2, and CD1d+ CD23− IgM+ IgD− marginal zone B cells. Interestingly, all these B cell subpopulations have been described as potential Breg cells (47). We can rule out that IL-10–producing B cells after L. major stimulation are B1 cells because purification of B cells was done by negative selection using a mAb against CD43, a marker expressed on B1–B cells (48). In addition, using B1-depleted BALB/c mice, Babai et al. (49) have clearly demonstrated that B1–B cells do not contribute to the susceptibility of BALB/c mice to infection with L. major.

With respect to the phenotype of the IL-10–producing B cells after L. major stimulation, we cannot conclude whether these cells are T2 or MZ B cells. Interestingly, recent data clearly demonstrated that a distinct subset of CD19+ CD1d+ B cells have potent regulatory function (50). This was confirmed recently in the murine model of EAE. Indeed, adoptive transfer of splenic IL-10-producing CD1d+ CD5+ B cells (named B10 cells) normalized EAE in B cell-depleted mice (51), showing that IL-10 strongly suggests that these IL-10–producing B cells correspond to the described B10 cells.

The results reported in this study clearly indicate that IL-10–produced B cells by B cells that express CD5+ CD1d+ are able to modulate the differentiation of CD4+ T cells. The fact that IL-10–producing B cells express CD1d+ and CD5+ are either in vitro or in vivo can post infection with L. major strongly suggests that these IL-10–producing B cells correspond to the described B10 cells.

Disclosures

The authors have no financial conflicts of interest.

References


