IL-33 Activates B1 Cells and Exacerbates Contact Sensitivity

Mousa Komai-Koma, Derek S. Gilchrist, Andrew N. J. McKenzie, Carl S. Goodyear, Damo Xu and Foo Y. Liew

*J Immunol* 2011; 186:2584-2591; Prepublished online 14 January 2011;
doi: 10.4049/jimmunol.1002103
http://www.jimmunol.org/content/186/4/2584

Supplementary Material

http://www.jimmunol.org/content/suppl/2011/01/14/jimmunol.1002103.DC1

References

This article cites 48 articles, 29 of which you can access for free at:
http://www.jimmunol.org/content/186/4/2584.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
IL-33 Activates B1 Cells and Exacerbates Contact Sensitivity

Mousa Komai-Koma,* Derek S. Gilchrist,* Andrew N. J. McKenzie,‡ Carl S. Goodyear,* Damo Xu,* and Foo Y. Liew*

B1 B cells produce natural IgM and play a critical role in the early defense against bacterial and viral infection. The polyreactive IgM also contributes to the clearance of apoptotic products and plays an important role in autoimmune pathogenesis. However, the mechanism of activation and proliferation of B1 cells remains obscure. In this study, we report that IL-33, a new member of the IL-1 family, activates B1 cells, which express the IL-33 receptor α, ST2. IL-33 markedly activated B1 cell proliferation and enhanced IgM, IL-5, and IL-13 production in vitro and in vivo in a ST2-dependent manner. The IL-33–activated B1 cell functions could be largely abolished by IL-5 neutralization and partially reduced by T cell or mast cell deficiency in vivo. ST2-deficient mice developed less severe oxazolone-induced contact sensitivity (CS) than did wild-type (WT) mice. Furthermore, IL-33 treatment significantly exacerbated CS in WT mice with enhanced B1 cell proliferation and IgM and IL-5 production. Moreover, IL-33–activated B1 cells from WT mice could adoptively transfer enhanced CS in ST22/2 mice challenged with IL-33. Thus, we demonstrate, to the best of our knowledge, a hitherto unrecognized mechanism of B1 cell activation and IL-33 function, and suggest that IL-33 may play an important role in delayed-type hypersensitivity.

Peripheral B cells consist of B1 and B2 subsets, which can be distinguished by their origin, surface markers, unique anatomical locations, and distinct Ab production profile (1). Unlike conventional B2 cells, which are CD45Rlo, sIgMhi, sIgDlo, and CD11b−, B1 cells are CD45Rhi, sIgMlo, sIgDhi, and CD11b+. The B1 cell population, which accounts for ∼5% of the total B cell pool in mice, is largely found in the peritoneal cavity, intestine, and pleural cavities and rarely in the spleen or lymph nodes, which are the main residence of B2 cells. B1 cells can be further subdivided into B1a and B1b by the pan-T cell marker CD5, which is present on B1a but not B1b, the minor subset (2–4).

Follicular B2 cells efficiently respond to protein Ags and undergo IgH class switching and affinity maturation in a T cell–dependent manner. In contrast, B1 cells are effectors of the innate immunity against bacteria, viruses, and certain parasites and respond to carbohydrate Ags in a T cell–independent manner (5–8). Unlike B2 cells, B1 cells are long-lived, self-renewing, and spontaneously secrete natural IgM, which is generally of low affinity and polyreactive to a broader range of pathogens and self-Ags (1–3). Furthermore, whereas T cells show little help to B1 cell function, B1 cells contribute to T cell–mediated immunity. B1 cells are effective APCs for T cell activation (9, 10). B1-derived IgM is required for the initiation of Ag-specific T cell migration during delayed-type hypersensitivity (DTH) (11). Thus, by producing polyreactive IgM to certain self-Ags, B1 cells are also associated with autoimmune diseases (12, 13). Understanding the nature of selective expansion of B1 cells is therefore of fundamental importance to immunity. Whereas conventional B2 cells are well studied, the mechanisms contributing to B1 cell proliferation are not well understood. IL-5, IL-9, and IL-10 are the only cytokines that have been reported to contribute to the expansion of B1 cells (14–16). We now report that IL-33, a new member of the IL-1 family, is an activator of B1 cell proliferation and function.

IL-33 is the latest member of the IL-1 family and is expressed by a variety of tissue stromal cells predominantly in the skin, lung, and the CNS, suggesting that IL-33 may play a physiopathological role in these organs (17, 18). Unlike IL-1 and IL-18 of the family, the protein maturation process is not necessary for IL-33 bioactivity. Caspase 1-mediated cleavage, which activates pro–IL-1 and pro–IL-18 to their mature forms, inactivates IL-33 (19, 20). Pro–IL-33 also contains a DNA binding domain and facilitates chromosome binding in epithelial cells and may regulate gene function (18). However, IL-33 can be released when cells undergo necrosis but not apoptosis (19, 20). IL-33 may therefore operate in an autoimmune and paracrine fashion in immune responses. IL-33 signals via a heterogeneous receptor complex consisting of ST2 and IL-1RacP and mainly triggers the activation of NF-κB and MAPKs (21, 22). ST2 is selectively expressed on Th2 but not Th1 cells, and ST2 is directly associated with Th2 but not Th1 functions (23–25). Consistent with these findings, IL-33 enhances IL-5 and IL-13 production by polarized Th2 but not Th1 cell lines in vitro (17, 26). In vivo, administration of IL-33 into naive mice provoked type 2 cytokine synthesis, splenomegaly, and eosinophilia (17). Interestingly, IL-33 is also able to induce IgA and IgE production in naive mice and augment IgGl and IgG2a secretions in inflammatory diseases, suggesting that IL-33 signals may play an active role in B cell function and Ab isotype switch (17, 27, 28).
Data reported in this study demonstrate that ST2 is expressed on activated B1 but not B2 cells. IL-33 induces B1 cell proliferation, type II cytokine synthesis, and IgM production in vitro and in vivo. The effect of IL-33 is largely IL-5–dependent and partially T- and mast cell–dependent. ST2+/− mice developed less severe oxazolone (4-ethoxymethylene-2-phenyl-2-oxalolin-5-one, or oxa)–induced contact hypersensitivity (CS) compared with the wild-type (WT) mice, and administration of IL-33 during Ag priming exacerbated CS via B1 cells. Thus, IL-33 is a hitherto unrecognized B1 cell activator acting upstream of IL-5 and promotes B1 cell–mediated immunity.

Materials and Methods

Mice

BALB/c, BALB/c nude, C57BL/6 (all from Harlan Olac), and C57BL/6Web/Wsh mice (The Jackson Laboratory) were used. ST2+/− mice on the BALB/c background were generated as described previously (29, 30). Mice were kept at the Biological Services, University of Glasgow, in accordance with the U.K. Home Office guidelines.

Reagents

IL-33 proteins were produced and purified as previously described (31, 32). The purity of IL-33 was >97%. Endotoxin levels were <0.1 endotoxin units/μg protein (Cambrex). IL-33 was also obtained from BioLegend, with which we obtained similar results. All mAbs for cell surface markers (CD4, CD3, CD8, CD19, CD5, CD11b) and their appropriate isotype controls were purchased from BD Biosciences. ST2 mAb and isotype control were from BD Biosciences. Recombinant IL-5 was obtained from PeproTech, and anti–IL-5 Ab and rat IgG were from eBioscience. Oxa was control were from BD Biosciences. Recombinant IL-5 was obtained from BioLegend and the U.K. Home Office guidelines.

Mouse peritoneal and spleen cell preparation and culture

Mice were injected i.p. with IL-33 (2 μg/mouse) or PBS daily for 5–7 d. This protocol was chosen following extensive dose and time course studies, using IL-33 dose range from 0.1 to 5.0 μg per mouse per injection given on 1–7 consecutive days. This protocol was consistent with similar studies on IL-33 (17, 26, 28). One day after the last injection, peritoneal cell cultures were collected by peritoneal wash with 2 ml cold PBS and counted. Spleens were removed, weighed, and total cell count was determined by light microscopy in a hemocytometer. After cytospin, the cells were stained with Rapi-Diff solution (Bios Europe, Skelmersdale, U.K.) and differential cell counts carried out under light microscopy. Subsets of B cells were purified from naive or IL-33–treated mice by staining with anti–CD19–PerCp, anti–CD3–allophycocyanin, and anti–CD11b–PE (BD Biosciences) using a FACSaria (BD Biosciences). The purity of B cells was >99%. Whole peritoneal cell cultures or subsets of B cells (2 × 106 cells/ml) were cultured in RPMI 1640 with 10% FCS in the presence or absence of IL-33 (20–50 ng/ml) for 48 or 72 h at 37°C and 5% CO2. Cell proliferation was determined by [3H]thymidine incorporation and cytokine production by ELISA.

Flow cytometry

Aliquots of freshly harvested cells (3 × 106 cells/tube) were suspended in buffer containing 2% FCS and 0.02% sodium azide in PBS, preincubated with CD16/32 (BD Biosciences) for 30 min, and stained with specific FITC-, PE-, PerCP-, or allophycocyanin-conjugated mAb for 20–30 min. The isotype–matched Abs were included as controls. Cells were analyzed by FACScalibur using CellQuest software (BD Biosciences) or FlowJo (Coeleza, Olten, Switzerland).

Quantitative PCR

RNA was purified using an RNAeasy Mini kit (Qiagen). Total RNA was reverse transcribed using AffinityScript (Stratagene), and transcript levels normalized to TATA box binding protein (TBP). Quantitative PCR was performed using SYBR Green Master mix on a Prism 7900HT (Applied Biosystems). Absolute transcript numbers were measured against DNA standards for each transcript of interest. Primers used were: IL-1RAcP, forward 5′-ACC GAG CTC ACT TTG GAA CAG A-3′, reverse 5′-GCC GCG TCA GCA GCA CAA ATT C-3′; TATA box binding protein, forward 5′-TGC TGT TGG TTA TTG GTG C-3′, reverse 5′-AAC TGG CTT GTG TGG GAA AG-3′; ST2L, forward 5′-TGC CCG ACC TTC TTG AAA A-3′, reverse 5′-TGT TAC GTG GGC AAG ATG C-3′.

Cytokine and IgM measurements

Cytokine concentrations in serum and peritoneal washes were determined by ELISA or Luminex according to manufacturer’s instructions (BioSource, Invitrogen). Ab pairs were obtained for IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and IL-9 (Ray Biotech). IgM titers of individual mice were also detected by ELISA (BD Pharmingen).

Contact sensitivity

CS was carried out as described previously (11). In brief, mice were sensitized by topical painting on the shaved abdomen with 10 μl of 3% oxa in ethanol/acetone (4:1) on day 0. Some mice were injected i.p. daily for 5 consecutive days with IL-33 (2 μg/injection/mouse) or PBS from day 0. On day 6, CS responses were elicited by topical ear application with 10 μl 0.4% oxa in acetone/olive oil (1:1) on the left ear and acetone/olive oil alone on the right ear. Ear thickness was measured with a caliper (Kroeplin) before and after challenge. In the B1 cell transfer experiment, CD19+CD11b+ B1 cells were purified by cell sorting from total peritoneal cells of IL-33–treated mice (2 μg/mouse i.p daily for 5 d) as above. ST2+/− recipients were reconstituted i.p. with 3–4 × 106 B1 cells in 0.2 ml PBS. Mice were immunized 24 h later and challenged with oxa and injected with or without IL-33 as described above. Ear thickness was measured at regular intervals.

Histology

For histological assessment, mice were sacrificed at the end of experiment and the ears removed and fixed with 10% PBS-buffered formalin (Sigma–Aldrich). Sections (5 μm) were deparaffinized, rehydrated, stained with H&E (Sigma-Aldrich) and examined under light microscopy.

Statistical analysis

Analysis with a Student t test was applied to in vitro studies. Analysis between individuals in groups in vivo was by ANOVA followed by a Student t test. Results are expressed as mean ± SEM and are representative of at least two individual experiments. A p value <0.05 was considered significant.

Results

IL-33 enhances B1 cell proliferation in naive mice

We sought initially to determine the effect of IL-33 on cellular influx in the spleen and peritoneum. Naive mice were injected i.p. with IL-33 or PBS daily for 7 d and the cell populations in the spleen and peritoneal cavity quantified by flow cytometry and cytokinin. IL-33 significantly enhanced total cell numbers in both spleen and peritoneum in WT, but not ST2−/− mice, compared with the PBS controls (Fig. 1A). Although most innate cells express ST2, only lymphocytes and eosinophils were significantly increased by the IL-33 treatment (Fig. 1B). Among the lymphocyte populations we found that the numbers of CD19+ B cells and, to a lesser extent, CD4+ (but not CD8+) T cells were significantly increased by IL-33 in the peritoneal cavity (Fig. 1C). We then determined the subsets of B cells expanded by IL-33 by staining for CD19, CD5, and CD11b. The number of B1 cells (CD19+ CD11b−) was significantly increased whereas the number of B2 cells (CD19−CD11b−) was modestly elevated (Fig. 1D). The number of both B1a (CD19+CD11b−CD5+) and B1b (CD19+ CD11b−CD5−) populations was similarly increased (Fig. 1E). The increase in the number of peritoneal cell subsets was evident after 2 daily injections of IL-33 and continued to expand after five to seven injections (Supplemental Fig. 1). To confirm that IL-33 induced B1 cell proliferation in situ (rather than attracting B1 cell migration), BALB/c mice were given daily i.p injections of IL-33 or PBS. BrdU was administered i.p. on day 5. Mice were sacrificed on day 6 and the peritoneal cell subsets analyzed by FACS. The number of B1 cells (BrdU+CD19+CD11b+) was increased by more than 10-fold (26.9 versus 2.1%) by the treatment of IL-33 compared with PBS control (Supplemental Fig. 2). Similar results were obtained for the splenic cell populations (data not shown).
Concomitant with B cells proliferation, IL-33 also induced significantly elevated production of IgM (Fig. 1F), IL-5, and IL-13 (Fig. 1G) in a ST2-dependent manner. Other cytokines (IL-4, IL-6, IL-9, IL-10, IFN-γ, and TNF-α) were not affected (data not shown). These results therefore demonstrate that IL-33 treatment in vivo led to the proliferation of predominantly B1 cells with the accompanying increased production of IgM, IL-5, and IL-13.

To further determine the long-term effect of IL-33 on B1 cell expansion and IgM and IL-5 production in vivo, naive mice were given five daily i.p. injections of IL-33 and the number of B1 cells in the peritoneum counted 1 or 7 wk later. The number of B1 cells was markedly elevated 1 wk after the IL-33 treatment, and this was sustained until at least 7 wk after injection (Supplemental Fig. 3). IgM concentration in the peritoneal fluid was similarly maintained. IL-5 concentration in the peritoneum was increased 1 wk after IL-33 treatment but returned to the basal level by week 7 (Supplemental Fig. 3). These results suggest that IL-33 induces a sustained effect on B1 cell expansion and IgM production in vivo.

**IL-33 activates B1 but not B2 cells in vitro**

To investigate the differential influence of IL-33 on B cell subsets, we examined the effect of IL-33 on purified B1 and B2 cells in vitro. We first sorted B1 and B2 cells from WT mice treated with IL-33 or PBS and stained them with anti-ST2 Ab. Naive B1 cells expressed a low but detectable level of ST2, which was markedly increased following IL-33 treatment (Fig. 2A). In contrast, naive or IL-33–treated B2 cells expressed little or no ST2.

To assess whether the ST2 expression on B1 cells is associated with their response to IL-33, highly purified B1a, B1b, and B2 cells from IL-33–treated WT or ST2−/− mice were cultured with or without IL-33 for 72 h and the cellular proliferation and the production of IgM and cytokines were determined. IL-33 significantly increased the cellular proliferation of and IgM production by B1b cells and, to a lesser extent, B1a cells, but not B2 cells (Fig. 2B). IL-33 had no effect on cells from ST2−/− mice. IL-33 induced high levels of IL-5 and IL-13 production only in B1b but not in B1a or B2 cells (Fig. 2C), demonstrating a subtle differential impact of IL-33 on B1 cell subsets. IL-4 and IL-10 were not detected in the cultures (data not shown). Thus, IL-33 signaling is able to enhance ST2 expression in B1 cells but not B2 cells, and that further IL-33 stimulation led to the proliferation and IgM production of B1 cells and the secretion of IL-5 and IL-13 by B1b cells.

To investigate the mechanism of the differential effect of IL-33 on B1a and B1b cells, we determined the expression of ST2 and IL-1RAcP on these two populations of cells in the peritoneum of mice following five daily i.p. doses of IL-33. B1a and B1b cells expressed similar levels of ST2. In contrast, B1b cells expressed a significantly higher level of IL-1RAcP (Fig. 2D). These results may explain the subtle differences between B1a and B1b cells in their response to IL-33.

We then investigated the effect of IL-33 on naive B cells in vitro. We found that IL-33 alone had little or no effect on naive B cells in vitro (data not shown), probably due to the low level of ST2 expression by these cells (Fig. 2A). However, IL-33 markedly enhanced B1 cell proliferation in the presence of IL-5 (Supplemental Fig. 4). These results suggest that in vivo IL-33 might have initially acted on other cell types (such as T cells and mast cells) to produce IL-5 (26, 32), which subsequently increase ST2 expression on B1 cells. IL-33 can then activate these B1 cells to proliferate further.

**IL-33 activation of B1 cell function in vivo is IL-5–dependent**

IL-5 is required for the development and function of B1 cells and eosinophils (14, 33). We therefore determined the role of IL-5 in...
IL-33–mediated B1 cell expansion and IgM production in vivo. Naive mice were injected i.p. once with an anti–IL-5 neutralizing Ab or a control normal IgG followed immediately with 7 daily i.p. injections of IL-33. As expected, IL-33 increased the numbers of eosinophils and CD19+ B cells in the peritoneum (Fig. 3A). IL-5 neutralization significantly reduced the number of eosinophils and CD19+ B cells (Fig. 3A). Importantly, anti–IL-5 Ab abolished IL-33–induced B1 (both B1a and B1b) cell proliferation (Fig. 3B). Consistent with this finding, anti–IL-5 Ab also abrogated IL-33–induced IgM production in the peritoneum (Fig. 3C). Anti–IL-5 Ab had no effect on the modest level of B2 cell expansion. IL-13 neutralizing Ab had no effect on B1 cell proliferation (Supplemental Fig. 5). These results therefore demonstrated that the IL-
33–mediated B1 cell expansion and IgM production is IL-5–dependent.

We next investigated whether IL-5 alone can induce B1 cell proliferation in vivo. Mice were injected i.p. with five consecutive daily doses of IL-5 or IL-33 and the peritoneal cells harvested and counted. As expected, IL-33–treated mice contained large numbers of peritoneal B1a and B1b cells compared with PBS-treated mice. Although IL-5–treated mice contained a similar number of B1a cells as did IL-33–treated mice, they contained a significantly lower number of B1b cells than did mice treated with IL-33 (Fig. 3D). However, B1a and B1b cells from IL-5–treated mice cultured in vitro produced significant proliferation and IgM synthesis in the presence or absence of IL-33. This is likely due to the secretion by IL-5–treated B1 cells of IL-5, which induces the proliferation of B1 cells in an autocrine manner. Taken together, these results indicate that IL-5 is necessary but insufficient to induce an optimal expansion of B1b cells. These results also indicate that ST2-/- mice would have normal B1 cell response activated by mediators other than IL-33.

IL-33–induced B1 cell amplification is partially T cell- and mast cell-dependent

T cells and mast cells are the key IL-33–responsive cells that produce IL-5 in response to IL-33 (21, 26, 27, 32). We sought next to determine whether T cells and mast cells are also associated with the IL-33–mediated B1 cell proliferation. Nude mice (which lack T cells) and WT BALB/c mice were injected with IL-33 or PBS. Peritoneal B1 cell counts and IL-5 and IgM concentrations were determined as above. IL-33 induced significant levels of B1 cell proliferation and IL-5 and IgM production in nude mice (Fig. 4A, 4B). However, the degree of B1 cells, and particularly B1b cells, proliferating in the nude mice was significantly lower than that in the WT mice (Fig. 4A). Furthermore, the concentration of IgM induced by IL-33 in the nude mice was also significantly reduced compared with that of the WT mice (Fig. 4B). Although nude mice may not be totally devoid of all T cells, we have elected not to use Rag-/- mice, which are also B cell deficient and that are essential for the read-out in our system. Even with this caveat, T cells are clearly involved with IL-33–associated B1 cell proliferation.

To determine whether mast cells also contribute to the in vivo effect of IL-33, mast cell-deficient (C57BL/6ShibWshb) and control WT C57BL/6 mice were injected with IL-33 or PBS. IL-33 induced similar levels of B1 cell proliferation and IgM production in the peritoneum of mast cell-deficient and WT mice (Fig. 4C, 4D). However, the concentration of IL-5 induced by IL-33 was significantly lower in the mast cell-deficient mice compared with that of the WT mice (Fig. 4D).

Taken together, these results show that depletion of either T cells or mast cells alone partially reduce IL-33–induced B1 cell activities, indicating that both T cells and mast cells contribute to the IL-33–induced B1 cell proliferation and the accompanied IL-5 and IgM production.

IL-33 exacerbatens CS

Hapten-induced CS is a classic T cell-mediated DTH, in which B1 cells play a critical role by producing hapten-specific IgM and initiating the recruitment of hapten-specific T cells (11). Because IL-33 is abundantly expressed in the skin (17) and IL-33 induced B1 cell proliferation, we reasoned that IL-33 may play a role in CS. WT and ST2-/- mice were primed with oxa on the skin of the abdomen. The mice were challenged on the ear with oxa 6 d after priming and the increase in ear thickness was measured at regular intervals for 48 h. As expected, oxa induced significant levels of CS, which peaked at 1 and 24 h (11). ST2-deficient mice developed significantly less severe CS compared with the WT mice at 24 h but not at 1 h (Fig. 5A), indicating an endogenous role of IL-33/ST2 signaling in DTH. We then investigated whether IL-33 could affect the induction of CS. BALB/c mice were primed with oxa as above and then treated with IL-33 i.p. for 5 consecutive days before being challenged with oxa on the ear on day 6. The severity of CS was significantly increased by the treatment with IL-33 at 24 h after challenge (Fig. 5B, Supplemental Fig. 6). The IL-33–mediated CS exacerbation was accompanied by elevated level of oxa-specific IgM Ab (Fig. 5B). Skin histology analysis showed that IL-33 increased oxa-induced inflammatory cell infiltration in the dermal and subdermal area of skin (Fig. 5C). IL-33 also significantly increased the number of total cells, B1 cells, and B1b cells in the peritoneum and the spleen (data not shown) in the oxa-primed and challenged mice (Fig. 5D). In contrast, the number of B1a cells was not significantly increased further by IL-33 treatment (Fig. 5D). These results therefore demonstrate that IL-33 plays an endogenous role in CS and that IL-33 can exacerbate CS, likely via the induction of B1b cells and the production of IgM.
IL-33 exacerbates CS via B1 cells

To directly determine whether the pathogenic effect of IL-33 on CS is mediated by B1 cells, ST2−/− mice were reconstituted i.p. with B1 cells (3–4 × 10^6) purified (>99%) from IL-33–treated donor mice. The recipient mice were primed (1 d after cell transfer) with oxa and treated i.p. for 5 consecutive days with IL-33 or PBS. All mice were challenged on day 6 with oxa on the ear as above. IL-33–primed B1 cells significantly increased the CS in the ST2−/− recipients at 24 h (Fig. 6A). Because the only cells that could respond to IL-33 in the recipient mice were the donor WT B1 cells, these results therefore demonstrate that IL-33–primed B1 cells are likely responsible for the IL-33–induced exacerbation of delayed CS. The exacerbated CS in the ST2−/− mice given IL-33–primed B1 cells and challenged with IL-33 was accompanied by significantly more total peritoneal cells and B1 cells than mice not given the B1 cells (Fig. 6B). Cellular analyses show that the number of B1a and particularly B1b cells was significantly expanded (Fig. 6C). Consistent with this observation, IL-33–primed B1 cells also markedly increased the production of IgM in the ST2−/− recipients in response to IL-33 treatment (Fig. 6D). ST2−/− mice given IL-33–activated B1 cells without further IL-33 treatment developed

FIGURE 5. IL-33 exacerbates CS. A, WT and ST2−/− mice were primed with oxa or carrier alone and challenged with oxa on day 6. Ear thickness was measured at 1 and 24 h and expressed as means ± SEM (n = 6 mice/group), and data are representative of two experiments. *p < 0.05. B–D, BALB/c mice were primed on the skin of the abdomen with oxa and injected i.p. with IL-33 or PBS for 5 consecutive days as described in Materials and Methods. Control mice were not primed or treated with IL-33. All mice were challenged on the skin of the ear with oxa on day 6 and ear thickness was measured. B, Ear thickness at 24 h after challenge is shown. There was no difference in the ear thickness between the groups treated with IL-33 or PBS at 1 h after challenge (data not shown). The serum IgM response at 24 h after challenge was also shown. C, Representative histology of the ear of the mice at 24 h after challenge is shown (H&E, original magnification ×10). D, Differential cell counts in the peritoneal lavage were also determined at 24 h after challenge. Data are means ± SEM and are pooled from two experiments (n = 10 mice/group). *p < 0.05, **p < 0.01.

FIGURE 6. IL-33–activated B1 cells exacerbate CS. B1 cells were sorted from the peritoneum of WT mice treated previously with IL-33 (5 consecutive days) and transferred i.p. into ST2−/− mice. The recipients together with WT mice were then primed 24 h later with oxa and injected i.p. with IL-33 or PBS for 5 consecutive days as described in Fig. 5. All mice were challenged on the ears and the ear thickness (A) was measured at 1 and 24 h after challenge. B and C, Total, B1, B1a, and B1b cell counts were also determined at 24 h. D, IgM titer in the serum and peritoneal fluid measured was by ELISA. Data are means ± SEM and are representative of two experiments (n = 6 mice/group). *p < 0.05, **p < 0.01.
a modestly enhanced CS when primed and challenged with oxa compared with mice not given the B1 cells (Supplemental Fig. 7), reflecting probably the presence of endogenous IL-33 in the recipients and that this low level of IL-33 could act on the donor WT B1 cells. Thus, IL-33 expands B1 cells, leading to elevated IgM synthesis, which then exacerbates CS. This series of events may account for the delayed effect of the transferred B1 cell in CS, that is, the CS-exacerbating effect of B1 cells plus IL-33 was observed at 24 h but not 1 h after cell transfer (Fig. 6A).

Discussion

Data reported in this study demonstrate that IL-33 is an activator of B1 B cells, leading to rapid and sustained B1 cell proliferation and IgM production that can promote CS. Because the IL-33 receptor ST2 is expressed on activated B1 cells and not B2 cells, ST2 may also be a novel marker to distinguish B1 cells from B2 cells.

The mechanism by which IL-33 preferentially activates B1 cells is at least via two pathways (Fig. 7). Several cell types, including mast cells, CD34+ hematopoietic progenitor cells, eosinophils, and Th2 cells, express ST2 (17, 21, 23, 34). Upon engagement with IL-33, these cells produce, among other mediators, IL-5, which could induce B1 cell proliferation and IgM synthesis. This notion is consistent with our observation that mast cells and T cells are partially involved in the IL-33–mediated B1 cell activation. IL-33 can also directly activate naive B1 cells, which express a low, but consistently detectable, level of ST2, leading to the synthesis of IL-5 that can act in an autocrine manner to further expand B1 cells. In both pathways, IL-5 is likely the key intermediate, as blocking with anti–IL-5 Ab completely abrogated IL-33–mediated B1 cell expansion and IgM production.

IL-33 is able to induce a relatively modest level of B2 cell proliferation in vivo (Fig. 1D). This is consistent with previous reports that IL-33 signals induce IgE and IgA in naive mice (17) and that IL-33 provokes collagen-specific IgG1 and IgG2a production in collagen-induced arthritis (27). Our finding suggests that the effect of IL-33 on B2 cells is likely to be indirect and perhaps via cytokines induced by IL-33. IL-5 also plays a role in B2 cell function and proliferation (35). Because anti–IL-5 Ab appears to have little influence on IL-33–induced B2 cell expansion (Fig. 3B), it is likely that IL-33 may amplify B2 cells via other cytokines concurrently induced by IL-33 in vivo.

It is intriguing that B1 cells but not B2 cells expressed ST2. This is reminiscent of the selective expression of ST2 on Th2 but not Th1 cells (23). As for Th1 and Th2 differentiation (36–39), the cytokine requirements for B1 and B2 cell development are also distinct (14, 16, 40). It is likely that ST2 expression is conditioned by the cytokines that polarized the cells during the developmental stage of cell lineages. Although both B1a and B1b cells express similar density of ST2, B1b cells express higher levels of IL-1RAcp than do B1a cells. This difference may account for the higher responsiveness of B1b cells to IL-33 than B1a cells in terms of cellular proliferation and IL-5 and IL-13 production. B1a cells spontaneously secrete IgM, which provides a first line of defense against certain encapsulated bacteria such as Streptococcus pneumoniae. In contrast, Ab produced by B1b cells is induced and has a role in providing long-term protection (8, 41–43). This is consistent with our finding that IL-33 induced a sustained B1b cell proliferation and IgM synthesis in mice at least up to 7 wk after treatment.

Using a CS model, we demonstrated that ST2−/− mice developed less severe CS than did WT mice, and that IL-33 can exacerbate the allergic skin inflammation via B1 cells. The process is likely to involve the enhanced IgM response since B1 cell-derived hapten-specific IgM are critically required for the initiation of CS by forming an immune complex and triggering the recruitment of Ag-specific T cells (11). Furthermore, IL-5 is required for the elicitation of CS by enhancing B1 and eosinophil functions (44). Our results therefore provide an upstream event by which B1 cells are activated for CS. However, B1 cells are also effective APCs. Thus, IL-33 administered during Ag priming may also enhance Ag-specific adaptive immunity by increasing the Ag presentation ability of B1 cells in vivo. Note that although ST2-deficient mice developed attenuated CS compared with WT mice, showing an endogenous role of IL-33, nevertheless CS can be induced in ST2−/− mice, indicating that IL-33/ST2 signaling is not mandatory for CS induction. Our study is consistent with a recent report that showed that IL-33 is not required for the development of CS (45), but rather indicated that IL-33 can exacerbate CS. Such a situation may occur during increased IL-33 production resulting from inflammation or infections (27, 28, 32, 46).

The pathogenesis of CS is complex and not well understood. It clearly consists of innate and adaptive components. The principal targets of IL-33 are the innate cells, through which IL-33 influences the adaptive T and B cell immunities. It has been shown that B1a and B1b cells can both mediate CS (47). Our data are not inconsistent with this finding, in that the exacerbated CS observed in this study is mediated by the selective induction of B1b cells by IL-33. The immediate response in CS (1 h after oxa challenge) is likely induced by several mediators, in addition to IL-33, whose manifestation via IL-5 and IgM synthesis is likely to be delayed (24 h).

Taken together, data reported in this study provide, to the best of our knowledge, a hitherto unrecognized function of IL-33 in the induction of B1b cell response and the associated innate and adaptive immunity. IL-33 and ST2 are clearly detected and elevated in clinical infections and autoimmune diseases, and they are induced by inflammatory signals (27, 28, 32, 48). Selective ablation of IL-33 leading to diminution of B1 cell expansion may represent

![FIGURE 7. Schematic representation of the mechanism of IL-33–mediated B1 cell activation. IL-33 binds to ST2 expressed on a variety of cells, including CD34+ hematopoietic cells. These cells produce, among other mediators, IL-5, which is principally responsible for the proliferation of and enhanced IgM synthesis by B1 cells. IL-33 can also bind directly to naive B1 cells leading to increased IL-5 synthesis, which can act in an autocrine manner for B1 cell proliferation. Anti–IL-5 Ab can block (blunted arrow) B1 cell proliferation and IgM production.](http://www.jimmunol.org)
Supplemental Figure S1. Time course of IL-33-induced cellular proliferation in vivo. Groups of BALB/c mice were injected i.p. daily for 1-7 days with IL-33 (2 μg per mouse per injection). Mice were sacrificed 24 h after the last injection and the peritoneal cells analysed by FACS. B1 cells are defined as CD19<sup>+</sup>CD11b<sup>+</sup>. Data are mean ± SEM, and are representative of 2 experiments, n=5. *p<0.05, **p<0.01 compare to PBS control.
Supplemental Figure S2. IL-33 induces B1 cell proliferation in vivo.

BALB/c mice were injected i.p. for 5 consecutive days with IL-33 (2 μg) or PBS. Both groups of mice were also injected i.p. with Bromodeoxyuridine (BrdU, BD, 1.5 mg/mouse) on day 5. All animals were sacrificed on day 6 and peritoneal cells harvested and analysed by FACS. The cells were gated for CD19+ and stained for CD11b and BrdU. The proliferating B1 cells are CD19+CD11b+BrdU+. Data are representative of 5 mice per group.
Supplemental Figure S3. IL-33 induces sustained B1 cell proliferation and IgM production. BALB/c mice were injected i.p with 7 consecutive daily doses of IL-33 (2 μg / injection/mouse) or PBS (open column). Mice were sacrificed 1 or 7 weeks after the last injection. (A) Peritoneal cells were analysed for B1a and B1b cells. (B) Peritoneal fluid was assayed for the concentrations of IL-5 and IgM. Data are mean ± SEM, n=5 and are representative of 2 experiments. *p<0.05, **p<0.01 compare to PBS control.
IL-33 induces naïve B1 cell proliferation in vitro in the presence of IL-5. Peritoneal cells from naïve BALB/c mice were harvested and sorted by FACS Aria into B1 and B2 cells and cultured in vitro with IL-33 (ng/ml) with or without IL-5 (10 ng/ml) for 72 h. Cell proliferation was then determined. Data are mean ± SEM, n=5 mice and are representative of 2 experiments. *p<0.05 compared with the group with IL-5 alone.
Supplemental Figure S5. IL-33-induced B cell proliferation and IL-5/IgM production were independent of IL-13.

BALB/c mice were injected i.p. with 50 μg of a neutralising anti-IL13 IL-13 antibody (R&D system) or control normal IgG, followed immediately by 7 daily i.p. injection of IL-33 (2 μg/injection). Peritoneal lavage was then analysed for B cells counts (A), and IL-5/IgM production by ELISA (B). Data are mean ± SEM, n=5.
Supplemental Figure S6. Time course of IL-33-induced exacerbation of contact sensitivity (CS). BALB/c mice were sensitized by topical painting on the shaved abdomen with a total of 100 μl of 3% oxa in ethanol/acetone (4:1) on day 0. Mice were injected i.p. daily for 5 consecutive days with IL-33 (2 μg/injection/mouse) or PBS from day 0. On day 6, CS responses were elicited by topical ear application with 10 μl of 0.4% oxa in acetone/olive oil (1:1) on the left ear and acetone/olive oil alone on the right ear (control). Ear thickness was measured with a caliper (Kroeplin) at regular intervals. Data are mean ± SEM, and are representative of 2 experiments, n=5, *p<0.05.
Supplemental Figure S7. IL-33-activated B1 cells exacerbate CS.

B1 cells were sorted from the peritoneum of WT mice injected previously with IL-33 (2 μg, 5 consecutive days) and transferred i.p. (3x10^6 cells) into ST2^-/- mice. The recipient were then primed 24 h later with oxa and injected with IL-33 (2 μg) or PBS for 5 consecutive days. All mice were challenged on the ears and the ear thickness measured 24 h later. Total peritoneal cell counts were also determined at 24 h and the IgM antibody titre in the serum measured by ELISA. Data are mean ± SEM, n=4. *p<0.05, **p<0.01.