Mac1 Positive Cells Are Required for Enhancement of Splenocytes Proliferation Caused by Bisphenol A

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We examined the effects of bisphenol A (BPA) on immune cells and it was shown that BPA upregulated the proliferation of murine splenocytes stimulated with Concanavalin A (ConA). The upregulating effects of BPA were removed with depleting Mac1+ cells from the splenocytes. This study provides evidence for the first time that Mac1+ cells were required for enhancement of splenocytes proliferation caused by bisphenol A.

Key words: bisphenol A; xenoestrogen; splenocytes; proliferation; Mac1

Environmental estrogens, also called xenoestrogens (XEs), are varied chemical compounds suspected to play a causative role in destabilization of sexual development in wildlife species and human. It is hypothesized that they bind to estrogen receptors and mimic estrogenic actions. But this mechanism is still a matter of debate.1,2) It is said that sex hormones have regulatory effects not only on the genital system but also the immune system.3,4) Therefore, it was suggested that XEs could modulate the immune system. Actually, there are some reports that XEs might modulate the immuno-response as well as hormonal systems.4,5)

Bisphenol A (BPA) is widely used as a material for polycarbonate resins and epoxy resins, and it was found to contaminate canned foods from the inner coating.6,7) BPA is thought to be one of the members of XEs,8,9) since some reports indicated that BPA showed estrogenic effects on reproductive cells. Added to these, some reports indicated exposure to BPA can affect immune responses.10–13) But the effects of BPA on the immune system are still unclear. Then, we investigated the immunologic effects of BPA and its mechanism.

Murine splenocytes from five to seven week-old BALB/c female mice (Charles River Japan, Inc., Tokyo, Japan) were treated with 2.5, 5, 10, or 20 μmol/l of BPA (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and 1 μg/ml of Concanavalin A (ConA, Sigma, St. Louis, MO) for up to 72 h. BPA was dissolved in dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a concentration of 25600 μmol/l, stored at −30°C, and diluted with the medium described below immediately before use. The medium for cell culture was RPMI 1640 (Nipro, Osaka, Japan) containing 100 U/ml penicillin, 100 μg/ml streptomycin, 5 × 10−5 mol/l 2-mercaptoethanol, and 10% fetal calf serum (FCS, ICN Biomedicals Inc. Irvine, CA). Step-by-step dilution was with the medium containing DMSO to keep the final DMSO concentration (0.156%). We had checked out that DMSO hardly influences reactivity of splenic cells at this concentration. Cell viabiliity was measured using a cell viability assay kit (Nacalai Tesque, Kyoto, Japan). Subsets of cultured splenocytes were stained with anti-CD3 mAb (clone: 145-2C11) conjugated fluorescein isothiocyanate, anti-CD16/32 mAb (clone: 93) (eBioscience, San Diego, CA), and propidium iodide (Sigma, St. Louis, MO) and analyzed with FACSort System (Becton Dickinson Immunocytometry Systems).

Exposure of splenocyte to BPA resulted in enhancement of splenocytes proliferation (Fig. 1 hatched bars) in a dose-dependent manner in the presence of ConA. Without ConA stimulation, BPA solely did not cause proliferation of splenocytes (data not shown). The results of the flowcytometric analysis confirmed that the hyper-proliferating cells in splenocytes under the existence of BPA were not CD3-negative cells, but CD3-positive cells (Fig. 2). These facts suggested that BPA enhanced activation of T cells with ConA.

The splenic T cells were cocultured with splenic feeder cells, and stimulated with ConA and BPA. Feeder cells were cultured with ConA and BPA, they were not clearly upregulated for the cell proliferation (Fig. 1 open bars). This result suggested that BPA did not upregulate the activation of T cell directly.

Therefore, we investigated which cells of splenocytes took part in enhancement of T cell proliferation by BPA. The splenic T cells were cocultured with splenic feeder cells, and stimulated with ConA and BPA. Feeder cells...
were Thy1–/C0 or Mac1–/C0 splenocytes that were separated with anti-Mac1 and anti-Thy1 MACS microbeads. In the case of Thy1–/C0 splenocytes, splenic T cells showed the enhancement of cell proliferation by BPA (Fig. 3). However, when the feeder cells were Thy1–/C0 Mac1–/C0 splenocytes, the enhancement of splenocytes proliferation with BPA was canceled. In addition, when the splenic T cells were cocultured with splenic Mac1+ cells in the presence of ConA, the enhancement of cell proliferation with BPA was recovered.

The major portion of Mac1+ splenocytes are macrophages. Macrophages, which play key roles in immunity, are dominant cells that modulate T cell function with their surface molecules and cytokines production. It was thus suggested that macrophages participated in the enhanced activation of splenic T cells with BPA. The concentrations of BPA used in these experiments were higher than those to which humans and animals are assumed to be exposed under natural conditions. But, recently it is becoming apparent that various types of macrophages exist and play various roles in immune and genital systems.14,15) Macrophages in genital organs may be affected by BPA even at lower concentrations than those used in this study and modify fecundity.

We could indicate in this study for the first time that Mac1+ splenocytes participated in the enhancement of

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Fig. 1. Proliferation of Splenocytes and Splenic T Cells with ConA in the Presence of BPA.

Mouse whole splenocytes or splenic T cells separated from whole splenocytes with MACS system (2 x 10⁶ cells/well) were treated with 0 (control), 2.5, 5, 10, or 20 μmol/l of BPA and 1 μg/ml of ConA for up to 72h. Cell viability, which is expressed by the mean values with SD for duplicate cultures, was measured using the cell viability assay kit. Data on whole splenocytes (hatched bars) and T cells (open bars) shown here are a representative of six and four independent experiments, respectively. Statistical comparisons were done using Student’s two-tailed t test. **Significantly different compared to control (p < 0.01).

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Fig. 2. Numbers of T Cells and Non-T Cells in Cultured Splenocytes in the Presence of ConA and BPA.

Mouse splenocytes (2 x 10⁶ cells/well) were cultured with 0 (control) or 20 μmol/l of BPA in the presence of 1 μg/ml of ConA in 24-wells plates. After 72 h of culture, recovered cells were counted and stained with anti-CD16/32 mAb (for blocking non-specific staining), anti-CD3 mAb conjugated fluorescein isothiocyanate, and propidium iodide (2.5 μg/ml). Flow cytometry was done using a FACSort machine (Becton Dickinson Immunocytometry Systems). Dead cells were excluded by gating propidium iodide positive subsets for analysis. Data were expressed as mean±SD (n=3). Statistical comparisons were done using Student’s two-tailed t test. **Significantly different compared to control (p < 0.01).

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Fig. 3. Cancellation of the BPA Effect by Deletion of Mac1+ Cells from Splenocytes.

Splen ic T cells (Thy1+) and feeder cells (Thy1–, Thy1–Mac1– or Mac1+) were prepared from whole splenocytes with the MACS system. The splenic T cells (0.5 x 10⁶) were cocultured with the splenic feeder cells (1.5 x 10⁷) in the presence of 1 μg/ml ConA and 0 (control) or 20 μmol/l BPA in a 96-well plate for 72h, and cell viability was counted. Cell viability data represent the mean values and SD for duplicate cultures. Statistical comparisons were done using Student’s two-tailed t test. **Significantly different compared to control (p < 0.01). Data of control (open bars) and BPA added (hatched bars) shown here are a representative of four independent experiments.
activation of splenic T cells with BPA in the presence of ConA. BPA actions on Mac1⁺ cells should be further investigated.

References


