

Possible Roles of B1 Cells and Environmental Estrogens (Endocrine Disruptors) in the Development of Autoimmune Diseases

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ABSTRACT

Autoimmune diseases as well as type-I allergic diseases have markedly increased in the past 30 years. Environmental estrogens or endocrine disruptors are possibly involved in the etiology of the increase in autoimmune diseases as one of environmental factors. In aged BWF1 mice, a murine model for SLE, B lymphocyte chemoattractant (BLC/CXCL13) is ectopically and highly expressed in target organs such as the thymus and kidney. B1 cells, a specialized cell population that are distinguished from conventional B cells (B2 cells) by their origin, cell surface phenotype, unique tissue distribution, self-reactivity, etc., preferentially migrate towards BLC. Aberrant B1 cell trafficking to the target organs may result in activation of autoreactive CD4 T cells, autoantibody production, and impaired mucosal immunity in the gut during the development of SLE. Interestingly, B1 cells show a higher sensitivity to environmental estrogens than conventional B (B2) cells to produce autoantibodies. Thus, B1 cell can be a useful target for evaluating the pathological significance of environmental estrogens in the development of autoimmune diseases.

KEY WORDS

autoantibody, B cell, chemokine, environmental estrogens, SLE

INTRODUCTION

A recent epidemiological study has revealed that approximately one third of the Japanese population is suffering from type-I allergic diseases including bronchial asthma, atopic dermatitis and allergic rhinitis. Environmental rather than genetic factors are likely involved in the marked increase in allergic diseases. In addition to environmental factors which directly affect the dose of allergens, it is pointed out that endocrine disruptors (ECDs) or environmental estrogens may be involved in the marked increase in allergic diseases.

It is well established that females have stronger immune responses than males. This phenomenon of gender-biased immune capability is largely attributed to the effects of sex hormones on the immune system.^{1,2} Estrogen regulates the synthesis of serum and

uterine IgM, IgA, and IgG and enhances antibody production to several non-self and self antigens.^{3,4} It is also well known that women are more susceptible to autoimmune diseases than men. There is compelling evidence, both in human and animals, that sex hormones play a major role in autoimmune diseases, particularly in lupus.⁵⁻⁷ The course of many autoimmune diseases is markedly affected during periods of physiological fluctuation in sex hormones (that is, pregnancy or menopause). Since many endocrine disruptors possess estrogenic activity, it is likely that the effect of environmental estrogens, if any, would appear on the gender-biased diseases such as autoimmune diseases.⁸

On the other hand, another epidemiological study has demonstrated that autoimmune diseases as well as type-I allergic diseases have been markedly increasing for the past 30 years (Fig. 1).⁹ It is generally

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Received 23 March 2005.

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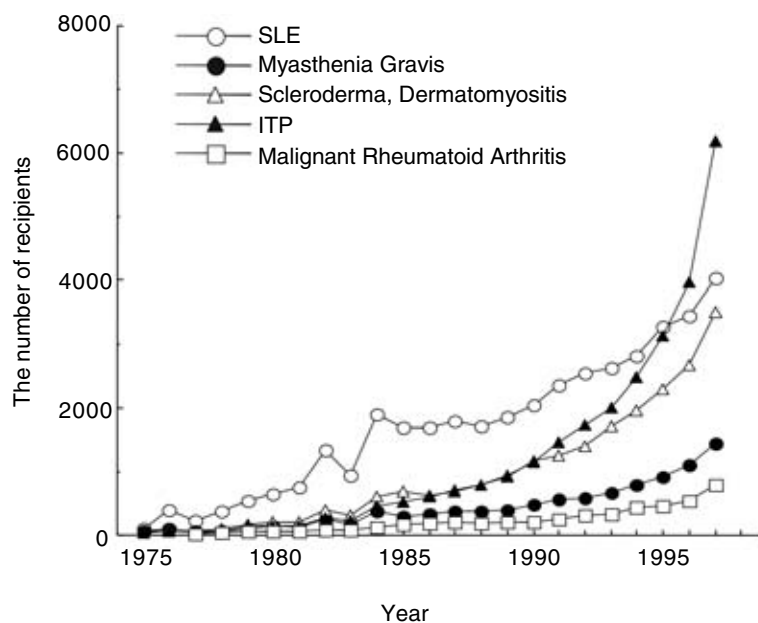


Fig. 1 Marked increase of autoimmune diseases in Japan. Changes from.⁹ The numbers of recipients of pension for intractable diseases including SLE (○), Myasthenia gravis (●), Dermatomyositis + Scleroderma (△), ITP (▲), malignant rheumatoid arthritis (□) have been increased for the past 30 years.

believed that autoimmune diseases are caused by the breakdown of immunological tolerance although the precise mechanism remains unknown. SLE is a systemic autoimmune disease characterized by production of a variety of IgG autoantibodies including anti-dsDNA Abs and massive immune complex deposition in the glomeruli of the kidney.¹⁰⁻¹² A marked mononuclear cell infiltration in the target organs including the kidney, thymus, and lung is another characteristic in SLE. Recent advances in molecular and biological analysis on chemokine/chemokine receptors have revealed pivotal roles of these proteins in inflammatory cellular infiltration.¹³ Chemokines and their receptors also play a pivotal role in linking innate immunity with acquired immunity through regulating dendritic cell trafficking.¹⁴ Th1/Th2 balance is regulated by the chemokine/chemokine receptor system and dysregulation of the balance is a general feature of the onset, progression, and prognosis of autoimmune diseases.^{15,16}

Using BWF1 mice, a murine model for SLE, we have demonstrated that aberrant B1 cell trafficking to the target organs due to ectopic expression of BLC/CXCL13 plays an important role in the pathogenesis of murine lupus.¹⁷⁻²¹

These findings in a murine model for SLE and the epidemiological study showing a marked increase in the number of SLE patients in Japan prompted us to examine the effects of environmental estrogens on

B1 cells. Our study has revealed that B1, but not B2 cells are susceptible to environmental estrogens and lead to the production of autoantibodies.²² Possible roles of environmental estrogens in the development of SLE and necessity of screening system specialized for autoimmune diseases are discussed.

B1 CELLS IN INNATE AND ACQUIRED IMMUNITY

B1 cells were first described in 1983 by Hayakawa *et al.*^{23,24} and suggested to be involved in the development of autoimmune diseases. B1 cells in the peritoneal cavity and spleen are increased in certain murine autoimmune models including NZB, NZB.H2bm12, moth-eaten mice and BWF1 mice. Likewise, elevated levels of B1 cells have been documented in patients with autoimmune disorders, notably in Sjögren's syndrome and rheumatoid arthritis.^{25,26} Furthermore, down-regulation/elimination of B1 cells by either administration of anti-IL-10 antibody^{27,28} or intraperitoneal injection of distilled water delays the onset and severity of lupus nephritis in BWF1 mice.²⁹

It is further demonstrated that B1 cells are involved in innate immunity by producing IgM antibodies against microbial antigens including phosphatidylcholine (PC), lipopolysaccharide (LPS), and α (1-3) dextran to eliminate blood borne-pathogens. IgM antibody produced by B1 cells also plays a role for prevention of certain viral infection.³⁰ On the other hand,

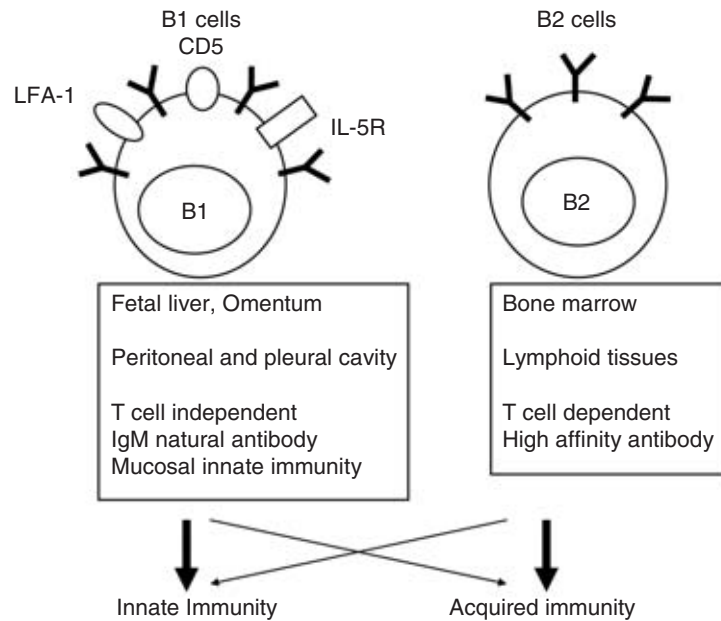


Fig. 2 Characteristics of B1 and B2 cells. B1 cells are distinct from conventional B cells (B2 cells) by their origin, tissue distribution, cell surface phenotypes, antigen specificity and so on.

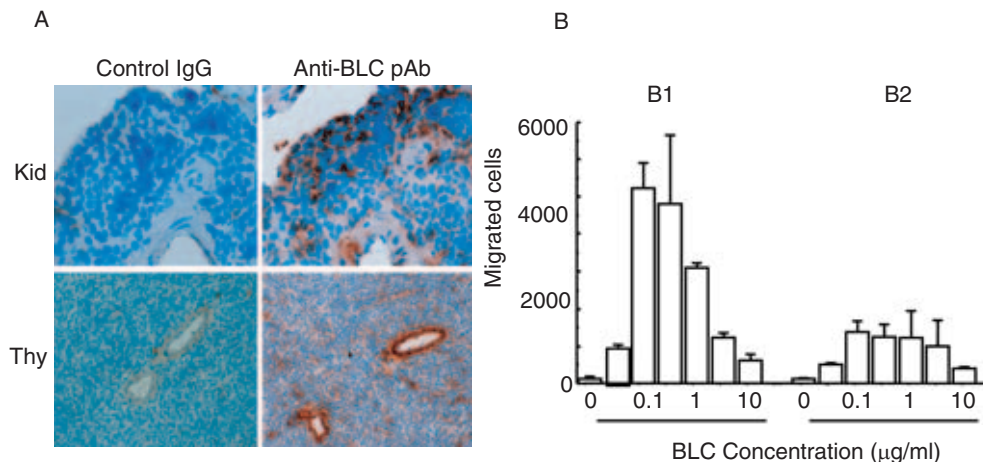


Fig. 3 Ectopic high expression of BLC in the target organs in aged BWF1 mice and preferential chemotaxis of B1 cells towards BLC. Changes from.¹⁷(A) Cryosections of kidney and thymus obtained from aged BWF1 mice were stained with goat anti-BLC polyclonal antibody (pAb) followed by biotinylated rabbit anti-goat pAb and HRP-labeled streptavidin. (B) Spleen or peritoneal cells from young BWF1 mice were stained for FITC-labeled CD5 and PE-B220 and sorted into B1 and B2 cells. Chemotaxis assay using ChemoTx plate (Neuro Probe, Gaithersburg, MD, USA) was performed according to manufacturer's instructions. Migrated cells were counted on a flow cytometer under the constant flow rate.

accumulating data suggest that B1 cells play an important role in mucosal immunity in the gut.³¹ Kroese *et al.*³² previously reported that approximately half of IgA⁺ B cells in the lamina propria in the intestinal mucosa were derived from B1 cells. It has been also demonstrated that B1 cells produce IgA antibody in

the presence of commensal microflora and contribute to prevention of systemic invasion by intestinal bacteria.³³

Another important feature of B1 cells is their potent antigen presenting ability.^{20,34} B1 cells stimulate alloreactive CD4 T cells as effectively as splenic DCs.

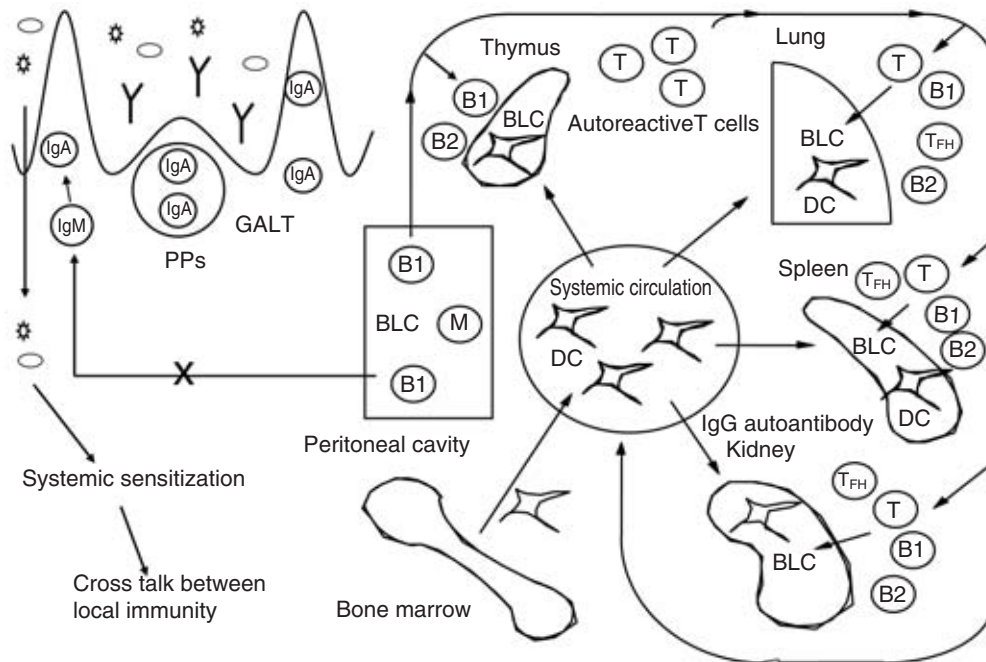


Fig. 4 SLE as a chemokine disease. Aberrant B1 cell trafficking due to ectopic high expression of BLC in aged BWF1 mice results in activation of autoreactive CD4 T cells in the thymus, IgG autoantibody production presumably in the presence of follicular helper T cells (T_{FH}), and impaired mucosal innate immunity in the gut. DC: dendritic cells, PPs: Peyer's patches, GALT: gut associated lymphoid tissues, M: macrophages

The potent antigen presenting ability of B1 cells is likely attributed to higher expression levels of accessory molecules including CD80, CD86, and ICAM-1 than that in B2 cells. Lanzavecchia *et al.*³⁵ reported that B cells could efficiently uptake the antigen through their surface immunoglobulin receptors, process, and then present it to specific T cells in a MHC-restricted manner. Since B1 cells often recognize self-antigens with low affinity and broad specificities, B1 cells would efficiently activate auto-reactive TCRs with low avidity. These findings suggest that B1 cells play a pivotal role both in innate and acquired immunity (Fig. 2).

ECTOPIC AND HIGH EXPRESSION OF B LYMPHOCYTE CHEMOATTRACTANT (BLC/CXCL13) AND PATHOLOGICAL SIGNIFICANCE OF B1 CELLS IN THE DEVELOPMENT OF SLE

B lymphocyte chemoattractant (BLC/CXCL13) is CXC chemokine which chemoattracts B cells and activated CD4 T cells expressing CXCR5, a receptor for BLC and is one of the homeostatic chemokines which is essential for lymphogenesis.^{36–38} We found that BLC is highly expressed in the target organs including the thymus and kidney in aged BWF1 mice¹⁷ (Fig. 3A). Interestingly, BLC chemoattracts B1 cells much more effectively than B2 cells probably due to

higher expression of CXCR5 on B1 cells than B2 cells¹⁷ (Fig. 3B). Ectopic and high expression of BLC in the target organs was attributed to the accumulation of mature myeloid DCs. The number of DCs in the circulation was increased around 5 months of age before the development of lupus nephritis and BLC was induced when peripheral blood DCs were cultured in the presence of $TNF-\alpha$ or $IL-1\beta$ for 3 days.¹⁸

Ansel *et al.*³⁹ demonstrated that BLC was essential for B1 cell homing to the peritoneal cavity. Ectopic high expression of BLC in the target organs in aged BWF1 mice resulted in the defective B1 cell homing to the peritoneal cavity and in preferential migration of B1 cells to the target organs.¹⁹ BLC up-regulation and B cell infiltration in the thymus was readily detectable around 5 months of age before the establishment of lupus nephritis. Furthermore, B1 cells activated autologous thymic CD4 T cells *in vitro* in the presence of $IL-2$.²⁰ The activation of thymic CD4 T cells by B1 cells is MHC class II-dependent and there is no TCR $V\beta$ skewing in activated T cells. On the other hand, CXCR5⁺CD4 T cells with similar phenotype to follicular helper T cells are increased in aged BWF1 mice and enhance IgG anti-DNA antibody production by B1 cells (unpublished data). These results suggest that aberrant B1 cell trafficking to the target organs due to ectopic and high expression of BLC/CXCL13 plays a pivotal role in the pathogenesis of

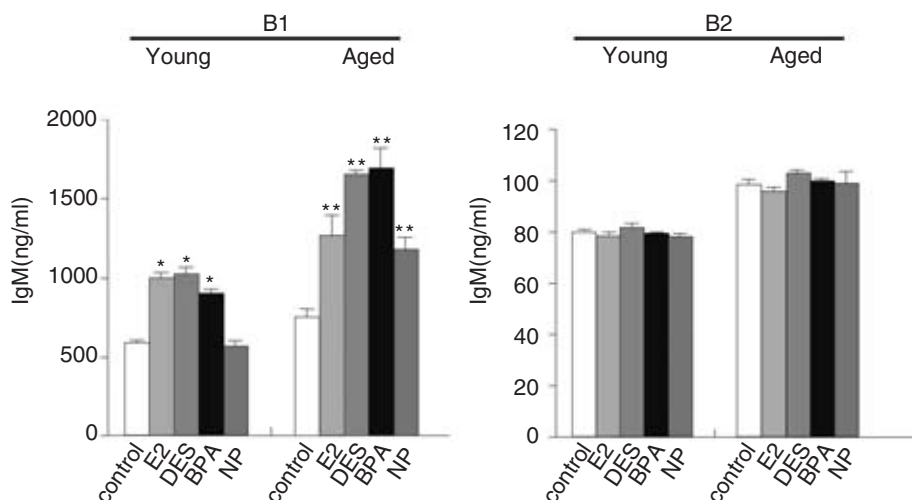


Fig. 5 Direct effect of environmental estrogens on IgM antibody production by B1 cells. Changes from.²² B1 or B2 cells obtained from young or aged BWF1 mice were cultured in the presence of E2 (100 nM), DES (100 nM), BPA (1 μ M) or NP (1 μ M) for 4 days, and the amount of total IgM in the culture supernatants were determined by ELISA. * $p < 0.05$, ** $p < 0.01$.

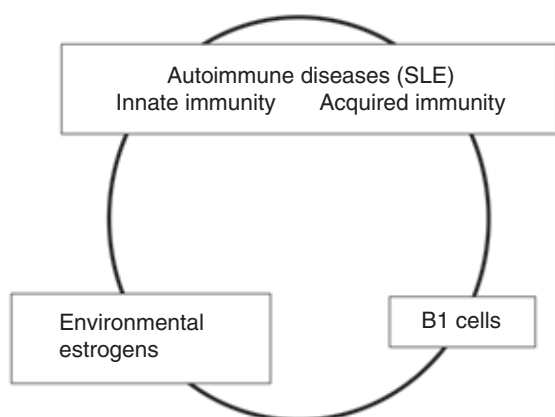


Fig. 6 B1 cells as a useful tool for evaluating the pathogenic effect of environmental estrogens on autoimmune diseases.

murine SLE (Fig. 4).

HIGHER SUSCEPTIBILITY OF B1 CELLS TO ENDOCRINE DISRUPTORS THAN B2 CELLS

Because SLE has been markedly increasing for the past 30 years and B1 cells play a pivotal role in the pathogenesis of murine SLE, it is tempting to speculate that B1 cells are possible targets for environmental estrogens. Indeed, IgM antibody production by B1 cells was significantly enhanced when cultured with estradiol (E2), Diethylstilbestrol (DES), or BisphenolA (BPA) (Fig. 5). Interestingly, B1 cells derived from aged BWF1 mice developing lupus nephri-

tis showed a higher response to environmental estrogens than those from young BWF1 mice. This is presumably due to higher expression of ER- α in B1 cells derived from aged BWF1 mice than those from young BWF1 mice. It was noted that environmental estrogens did not affect B2 cells at all. An enhancing effect of environmental estrogens including DES and BPA on autoantibody production was further confirmed *in vivo*. E2, DES, and BPA implanted subcutaneously in silastic tubes enhanced autoantibody production to bromelain-treated RBC. It was previously demonstrated that anti-Br-RBC Ab was mainly produced by B1 cells.⁴⁰ Implanted E2 and DES, but not BPA also enhanced IgG anti-dsDNA antibody production as well as IgG deposition in the glomeruli in the kidney. The failure of BPA to enhance IgG autoantibody production is likely attributed to weak estrogenic activity of BPA.⁴¹ The estrogen level in di-estrous female mice is around 20–30 pg/ml and increases to 100–200 pg/ml during estrus. It reaches to 5,000–10,000 pg/ml during pregnancy. Therefore, the serum levels of E2 in mice implanted with E2 silastic tubes are in the middle of those values between estrus and pregnancy. Long lasting release of BPA from implanted tubes was also observed although BPA concentration in the serum was much higher than that expected from environmental exposure in human population. Analysis of gene expression using DNA microarray will provide useful information that can be used to establish a screening system for environmental estrogens which may be involved in autoantibody production by B1 cells and to evaluate the pathological significance of those in the develop-

ment of autoimmune diseases.

CONCLUDING REMARKS

Environmental estrogens may possibly contribute to the marked increase in type-I allergic and autoimmune diseases. However, it is difficult to clarify the causality between environmental estrogens and diseases because it becomes evident only very slowly and indiscriminately. We should be ready to start a new strategy to protect human health from these kind of threats before it becomes too late. Hopefully, B1 cells will provide a good target to evaluate the biological significance of environmental estrogens in the development of autoimmune diseases (Fig. 6).

ACKNOWLEDGEMENTS

This work was supported by SORST (solution Oriented Research for Science and Technology) by Japan Science and Technology Corporation and LRI (The Long-ranged Research Initiative) by Japan Chemical Industry Association.

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