Lupus (2004) 13, 217–222
www.lupus-journal.com

REVIEW

Possible mechanisms of gender bias in SLE: a new hypothesis involving a comparison of SLE with atopy

I Sekigawa[1]*, T Naito[2], K Hira[3], K Mitsuishi[3], H Ogasawara[4], H Hashimoto[4] and H Ogawa[3]
[1]Department of Medicine, Juntendo University Izu-Nagaoka Hospital, Shizuoka, Japan; [2]Department of General Medicine, Juntendo University School of Medicine, Tokyo, Japan; [3]Department of Dermatology and Atopy Research Center, Juntendo University School of Medicine, Tokyo, Japan; and [4]Department of Internal Medicine and Rheumatology, Juntendo University School of Medicine, Tokyo, Japan

The prevalence of systemic lupus erythematosus (SLE) is far higher in females than in males, and numerous investigations of this gender bias have been performed from several perspectives. Sex hormones, particularly estrogens, may be significant in causing the gender discrepancy. This article discusses the possible importance of estrogens in regulating the expression of and responsivity to autoantigens in SLE and in atopic disorders, which are associated with hyperreactivity to exogenous antigens. Estrogens seem to play an important role in the overexpression of endogenous autoantigens, such as human endogenous retroviruses (HERV), and this may be related to the existence of a gender bias in the incidence of SLE but not atopy. *Lupus* (2004) **13,** 217–222.

Key words: atopic disease; DNA methylation; estrogen; human endogenous retrovirus; systemic lupus erythematosus

Introduction

The incidence of systemic lupus erythematosus (SLE) is well known to be far higher in females than in males, and there have been numerous studies concerning this issue in humans and in mouse models of SLE.[1] Sex hormones (especially estrogens) seem to be an important factor in this gender difference in the incidence of SLE. In fact, estrogen is thought to play a role in promoting immune responses, including the production of cytokines and antibodies.[2–4]

The pathophysiological mechanism of SLE may involve a combination of several activating or environmental factors (e.g., viral and bacterial infection, ultraviolet light and stress) with the predisposition of the host [e.g., genetic factors such as human leukocyte antigen (HLA) types, HERV and sex hormones]. Through a combination of these mechanisms, an increase in the expression of endogenous autoantigens (selfantigens) such as HERV and responsivity to these autoantigens may occur that plays a significant role in the etiology of SLE.[5–7] This hyperreactivity of SLE patients is considered to be partly regulated by impairment of epigenetic mechanisms (inheritable changes of gene expression that occur without a change in the DNA sequence), especially a decrease of DNA methylation levels.[7–9] In SLE patients, hyperresponsivity to exogenous nonself antigens (including viral and bacterial infections) is also observed, similar to that seen in patients with atopic disorders, which do not usually have a gender bias in incidence.[10] Sex hormones may participate in the development of such hyperreactivity in addition to impairment of DNA methylation.

From this view point, in order to promote investigation into the basis of the gender bias in SLE, we here review the role of sex hormones in the pathogenesis of SLE and discuss the possible participation of these hormones in the overexpression of and hyperresponsivity to autoantigens in SLE patients.

Gender discrepancy in SLE and the role of sex hormones

The female versus male (F/M) ratio of SLE patients is reported to range between 7 : 1 and 20 : 1.[1,11] In addition, the disease is more severe in female than male NZB × NZW F1 mice (B/W F1; a representative

*Correspondence: Iwao Sekigawa, MD, Department of Medicine, Juntendo University Izu-Nagaoka Hospital, 1129 Nagaoka, Izu-Nagaoka-cho, Tagata-gun, Shizuoka 410-2295, Japan. E-mail: isekigawa@mva.biglobe.ne.jp
Received 12 September 2003; accepted 10 February 2004

10.1191/0961203304lu1012ed

model mouse of human SLE).[12] The reasons for the gender bias of SLE appear to be complicated, but sex hormones (especially estrogens) may well be involved. For instance, fluctuation of SLE related dermatitis (such as the classic malar rash) with the menstrual cycle has been empirically observed in some female patients with SLE.[13]

Evidence regarding the role of sex hormones in the gender bias in the incidence of SLE is summarized in Table 1.[2–4] Estrogen receptors are expressed by many immune cells, such as thymocytes, CD4+ and CD8+ T cells, B cells, macrophages and endothelial cells. At physiological levels, estrogen principally acts as an enhancer of certain immune responses, while androgens and progesterone act as natural suppressors.[2] In fact, serum immunoglobulin levels and the humoral immune response are reported to be higher in female mice and humans compared with males.[14,15] In mouse models of SLE (such as B/W F1 and MRL/lpr mice), exacerbation of disease manifestations by administration of estrogen and improvement by administration of androgen are observed.[12,16–20] In human SLE patients, it has been reported that certain hormone therapies [such as hormone replacement therapy (HRT) in postmenopausal women and stimulation of ovulation] can induce exacerbation of the activity of SLE, while treatment with an exogenous androgen (dehydroepiandrosterone; DHEAS) results in modest amelioration of disease manifestations.[21–24] Flare-up of SLE commonly occurs during pregnancy (which causes hyperestrogenemia), as well as in the puerperium, though such exacerbations are usually mild.[25–27] The increase of endogenous steroids during late pregnancy may explain why the majority of women with SLE have a successful delivery and why improvement occasionally occurs in their clinical and laboratory manifestations.[3] *In vitro* experiments on mouse and human cells have revealed that estrogens can induce the expansion of autoreactive B and T cells and can magnify the B cell response to mitogens.[28–31] In SLE, the cytokine profile is known to be T helper (Th)-2 dominant.[32] Interleukin (IL)-6 and IL-10 (Th-2 cytokines) are the most potent activators of B cells, promoting both proliferation and immunoglobulin production,[33,34] and administration of IL-6 or IL-10 accelerates the development of SLE in B/W F1 mice, while anti-IL-6 or anti-IL-10 delays its onset.[35,36] Generally, estrogens stimulate the production of Th-2 cytokines (such as IL-4, IL-6 and IL-10), while antiestrogens promote the production of Th-1 cytokines (such as IL-2 and IFN gamma).[4,37–40] Certain reports have indicated that the effects of estrogen on the development of SLE in mice or humans could be mediated by changes in the number and affinity of estrogen receptors.[41,42] Thus, estrogens should be one of the factors that enhance SLE and may act as an accelerator for the underlying pathogenetic factors or events, although hormones alone are an insufficient explanation for the cause of SLE.

Effect of estrogens on expression of endogenous antigens such as HERV

Numerous reports have suggested a possible important role of HERV as autoantigen in the etiology of SLE through several mechanisms such as molecular mimicry between HERV and autoantibodies,[7] although this is still controversial and the precise role of these

Table 1 Evidence of the important role of sex hormones in the induction of SLE

Evidence	*References*
Gender differences of SLE and the immune response	
Higher incidence of SLE in females than males	1,11
More severe disease activity in females than males in a mouse model of SLE (B/WF1)	12
Higher production of immunoglobulins and stronger humoral immune responses in females than males (mice and humans)	14,15
Sex hormones and SLE in vivo	
Mice: Protective effect of androgens and enhancing effect of estrogens on SLE (B/WF1 or MRL/lpr)	12,16,17,18
Estrogen-induced enhancement of B cell activity and autoantibody production in normal mice	19,20
Humans: Exacerbation or onset of SLE after hormone replacement therapy or repeated cycles of ovulation therapy	21,22,23
Modest clinical improvement of SLE by exogenous DHEAS	24
Flare-up of SLE in pregnancy	25,26,27
Effects of estrogen on cellular immunity in vitro	
Development of autoreactive T and B cells through resistance to apoptosis in estrogen-treated mouse cells	20,28,29
Increase of immunoglobulin production or mitogen-mediated B cell responses by estrogen in human cells	30,31
Modification of cytokines by sex hormones	
Mice: Increase of IL-6 and IL-10 production *in vitro* by estrogen	4
Increase of IL-2 and IFN-gamma (Th-1) and decrease of IL-1, IL-10, and TNF-alpha (Th-2) production *in vivo* by antiestrogens	37,38
Humans: Increase of IL-6 by estrogen during the menstrual cycle	39
Close relationship between IL-4 secreting cell numbers and estrogen during the menstrual cycle, and closed relationship between IFN-gamma secreting cell numbers and DHEAS in men or premenopausal women	4,40

HERV remains unclear.[43] HERV account for approximately 8% of human DNA, although their transcription and translation are blocked by several interrupters such as termination codons, deletions, and methylation sites in normal people. Our recent results have indicated that transcription and translation of the gene for HERV clone 4-1 (which belongs to the HERV-K family) are markedly increased in SLE patients compared with normal controls, while serum autoantibodies to this HERV and expression of its antigens on lymphocytes are detected in SLE patients, but not normal controls.[7–9,44,45] In addition, synthetic peptides derived from HERV clone 4-1 can induce the immune abnormalities observed in SLE patients, such as T cell activation, cytokine production, and polyclonal B cell activation.[46]

The increased transcription of HERV clone 4-1 in SLE patients is partially regulated by epigenetic mechanisms, as is the case for other HERV.[8,9,47] Cytosine methylation of the regulatory sequences of DNA is an epigenetic mechanism that is associated with the inactivation of gene transcription, while hypomethylation promotes transcription. DNA methyltransferase (DNMT) is the enzyme family responsible for methylation of DNA and DNMT-1 is the first member of this family.[48] In normal individuals, the level of HERV clone 4-1 transcription is increased by demethylating agents (such as 5-aza-deoxycytidine: 5-aza C) that inhibit DNMT-1, and the level of DNMT-1 activity in SLE patients is lower than in normal controls.[8,9,48] The expression (especially transcription) of endogenous autoantigens such as HERV seems to be promoted by DNA hypomethylation, which is implied by low DNMT-1 activity.

Estrogens may increase the expression of endogenous autoantigens including HERV, especially via the enhancement of transcriptional processes. Supporting this possibility, the expression of HERV-K in breast cancer cells is reported to be enhanced by estrogen (estradiol) treatment,[49]and the transcription of some nuclear proto-oncogenes (c-myc, c-jun and c-fos) in animal cells and human cells is also increased by estrogens (especially 17 beta-estradiol; E2).[50,51] The precise mechanisms underlying these phenomena remain unclear, but estrogens may reduce DNA methylation through the inhibition of DNMT-1 activity.

Effect of sex hormones and DNA methylation on hyperresponsivity to antigens in SLE and atopy

Estrogens may promote responsivity to antigens through several mechanisms, such as enhancement of Th-2 cytokine production, as described above (Table 1). In addition, a low level of DNMT-1 activity seems to exert a great influence on hyperresponsivity to antigens (autoantigens) via several immune mechanisms, including expression of autoimmune related cell surface molecules (e.g., leukocyte function associated antigen-1; LFA-1) and production of cytokines and/or antibodies in patients with SLE.[48,52–56] A decrease of DNA methylation reduces the threshold for immune responses to antigenic stimulation and thus leads to hyperresponsivity. Atopic diseases (such as atopic dermatitis, asthma, allergic rhinitis and allergic conjunctivitis) are representative disorders that feature hyperresponsivity to stimulation by antigens, although such antigens are exogenous (allergens) and not endogenous (autoantigens), unlike in SLE.[10] A previous study indicated that IL-4-mediated IgE production by B cells is regulated through DNA hypomethylation.[57] Our recent preliminary study found low levels of DNMT-1 activity in patients with atopic dermatitis (especially those who had high serum IgE levels) as well as SLE patients. Thus, DNA hypomethylation appears to be associated with hyperresponsivity to allergens in atopic diseases such as atopic dermatitis.[10]

A gender bias in the incidence of atopic diseases has not been observed, but premenstrual exacerbation is well known to occur in women with atopic dermatitis (premenstrual syndrome). This worsening of skin lesions associated with the menstrual cycle may indicate the importance of female sex hormones such as estrogens in the development of such diseases.[58] The possible effects of DNA hypomethylation and estrogens on hyperresponsivity to and overexpression of antigens in SLE and atopy are summarized in Table 2.

Differences between SLE and atopy in the role of estrogen in the immune response

Allergic reactions to drugs such as antibiotics are widely known to occur in SLE patients.[59] However, several recent studies have indicated that the incidence of IgE-mediated and/or associated atopic diseases (including dermatitis, asthma, rhinitis and conjunctivitis) is lower in SLE patients than in normal controls.[60–62] This can be partly explained by immunological differences between atopy and SLE, although the cytokine profiles are similar in both (Th-2 dominant), probably reflecting hyperresponsivity to autoantigens or allergens.[10,62] The ratio of CD4+ to CD8+ T cells (CD4/CD8 ratio) among peripheral blood lymphocytes is decreased in SLE, but is increased in atopy, and this is thought to reflect the recognition of endogenous autoantigens (such as HERV) by CD8+ T cells in SLE versus recognition of exogenous allergens by CD4+ T cells in atopy.[10] This may also be concerned with the low incidence of patients with both typical SLE and atopy. In fact, the CD4/CD8 ratio is reported to be atypical in SLE

Table 2 Role of DNA hypomethylation and estrogens in the overproduction of autoantigens and hyperresponsivity to antigens in both SLE and atopy

	DNA hypomethylation		*Estrogen*	
Events	*SLE*	*Atopy*	*SLE*	*Atopy*
Overproduction of autoantigens through increased transcription	+ (7,8,9,47)	−	+ (49,50,51)	−
Hyperresponsivity to endogenous and exogenous antigens	+ (48,52,53,54)	+ (10,57)	+ (2,3,4)	+ (58,66)

Major (+) or minor (−) participation of DNA methylation or estrogen in the development of events in each disease. Numbers in parentheses are the main references to these events.

patients who have atopic dermatitis.[63] In contrast, the family history of atopy is increased in SLE patients compared with non-SLE controls,[60,62] indicating the existence of immunological and/or genetic similarities between the diseases. Shared immunological features seem to consist of a hyperresponsivity to antigens that is related to DNA hypomethylation and a similar cytokine profile (Th-2 dominant), as described above. Based on these similarities between SLE and atopy, there may be a difference in the orientation of antigen–antibody responses (targeting endogenous autoantigens in the former disease and exogenous allergens in the latter) between these diseases. Sex hormones such as estrogens may contribute to determining the direction of the immune response (Table 2). Thus, the overexpression of endogenous antigens such as HERV due to a mechanism promoted by female sex hormones may be related to the gender bias of SLE.

Immunological characteristics of male SLE

There have been few reports about the immunological and/or serological characteristics of male patients with SLE because of the small number of affected males compared with females. We recently reported that serum IgE levels are significantly higher in SLE patients than in normal individuals independent of disease activity, and that IgE levels are not related to the development of atopic diseases in SLE patients.[64] In our SLE group (12 males and 47 females), the percentage of patients with serum IgE levels higher than in normal control (normal ranges of IgE $\leqq$ 250 IU/mL) was much greater among male than female patients (58% in males versus 21% in females with SLE, $P = 0.011$ by the chi-squared test), and this was one of the major differences of routine laboratory data between males and females with SLE.[65] It has been reported that IgE production is promoted by DNA hypomethylation and is also enhanced by estrogens,[57,66] and that IgE production mediated by IL-4 is closely associated with the estrogen level.[4,40] Thus, high IgE levels in male SLE patients may reflect hyperreactivities (including overproduction of and hyperresponsivity to autoantigens) due to DNA hypomethylation that is not related to the influence of sex hormones. In women with postmenopausal onset of SLE, which occurs occasionally, similar findings may also occur although precise data are still insufficient to confirm this.[67]

Conclusion

We described a possible mechanism for the gender difference in the incidence of SLE, based on the reported data including our findings. Comparison of SLE and atopy suggests that estrogen may be related to hyperresponsivity to exogenous or endogenous antigens, mainly through promoting the production of Th-2 type cytokines in both diseases, and that DNA hypomethylation is also involved. In patients with SLE, but not those with atopy, estrogen also seems to contribute to the overproduction of endogenous autoantigens (mediated by increased transcription), in cooperation with DNA hypomethylation. This may be an important reason for a gender bias existing in SLE, but not atopy. In order to make this point clearer, the precise role of sex hormones (such as estrogens) and epigenetic mechanisms (such as DNA methylation) in the transcription/translation of endogenous autoantigens (including HERV) needs to be determined. Further investigation of male SLE or late onset SLE may also make an important contribution to elucidation of the reason for the gender bias of this disease.

While sex hormones alone are not responsible for the gender bias in the development of SLE, these hormones may promote other factors (genetic factors, infections, etc.) that trigger the disease and may modulate its course. Furthermore, we cannot neglect the possibility that factors other than sex hormones may contribute to the development of similarities and/or differences in clinical manifestation between SLE and atopy. However, findings obtained from investigations into this field should also contribute to further understanding

of the important role of sex hormones in human immunity beyond SLE.

Acknowledgement

This work was supported by the grants from The Institute for Environmental and Gender-specific Medicine, Juntendo University School of Medicine.

References

1 Lockshin MD. Sex ratio and rheumatic disease: excerpts from an institute of medicine report. *Lupus* 2002; **11**: 662–666.
2 Cutolo M, Sulli A, Seriolo B, Accardo S, Masi AT. Estrogens, the immune response and autoimmunity. *Clin Exp Rheum* 1995; **13**: 217–226.
3 Cutolo M, Sulli A, Villaggio B, Seriolo B, Accardo S. Relations between steroid hormones and cytokines in rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* 1998; **57**: 573–577.
4 Verthelyi D. Sex hormones as immunomodulators in health and disease. *Int Immunopharmacol* 2001; **1**: 983–993.
5 Perl A. Endogenous retroviruses in pathogenesis of autoimmunity. *J Rheumatol* 2001; **28**: 461–464.
6 Poris JL. Perspectives on the role of endogenous human retroviruses in autoimmune diseases. *Virology* 2002; **296**: 1–5.
7 Sekigawa I, Ogasawara H, Naito T, Kaneko H, Hishikawa T, Hashimoto H. Systemic lupus erythematosus and human endogenous retroviruses. *Mod Rheumatol* 2003; **13**: 107–113.
8 Okada M, Ogasawara H, Kaneko H *et al*. Role of DNA methylation in the transcription of human endogenous retroviruses in the pathogenesis of systemic lupus erythematosus. *J Rheumatol* 2002; **29**: 1678–1682.
9 Ogasawara H, Okada M, Kaneko H, Hishikawa T, Sekigawa I, Hashimoto H. Possible role of DNA hypomethylation in the induction of SLE: relationship to the transcription of human endogenous retroviruses. *Clin Exp Rheumatol* 2003; **21**; 733–738.
10 Sekigawa I, Yoshiike T, Iida N, Hashimoto H, Ogawa H. Allergic diseases in systemic lupus erythematosus: prevalence and immunological considerations. *Clin Exp Rheumatol* 2003; **21**: 117–121.
11 Beeson P. Age and sex association of 40 autoimmune diseases. *Am J Med* 1994; **96**: 457–467.
12 Siiteri PK, Jones LA, Roubinian JR, Talal N. Sex steroids and the immune system: I. Sex differences in autoimmune disease in NZB/NZW hybrid mice. *J Steroid Biochem* 1980; **12**: 425–432.
13 Yell JA, Burge SM. The effect of hormonal changes on cutaneous disease in lupus erythematosus. *Br J Dermatol* 1993; **129**: 18–22.
14 Ansar Ahmed S, Talal N. Sex hormones and the immune system: Part 2. Animal data. *Bailliere's Clin Rheum* 1990; **4**: 13–31.
15 Da Silva JAP. Sex hormones, glucocorticoids and autoimmunity: facts and hypotheses. *Annu Rev Immunol* 1994; **13**: 307–338.
16 Steinberg AD, Roths JB, Murphy ED, Steinberg RT, Raveche ES. Effects thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. *J Immunol* 1980; **125**: 871–873.
17 Walker S, Keisler LW, Caldwell CW, Kier AB, Saal FS. Effects of altered prenatal hormone environment on expression of autoimmune disease in NZB/NZW mice. *Environ Health Perspect* 1996; **104**: 815–821.
18 Norton SD, Harrison LL, Yowell R, Araneo BA. Administration of dehydroepiandrosterone sulfate retards onset but not progression of autoimmune disease in NZB/W mice. *Autoimmunity* 1997; **26**: 161–171.
19 Verthelyi D, Ansar Ahmed S. Characterization of estrogen-induced autoantibodies to cardiolipin in non-autoimmune mice. *J Autoimmun* 1997; **10**: 115–125.
20 Verthelyi D, Ansar Ahmed S. Estrogen increases the number of plasma cells and enhances their autoantibody production in nonautoimmune C57BL/6 mice. *Cell Immunol* 1998; **18**: 125–134.
21 Meier CR, Sturkenboom MC, Cohen AS, Jick H. Postmenopausal estrogen replacement therapy and the risk of developing systemic lupus erythematosus or discoid lupus. *J Rheumatol* 1998; **25**: 1515–1519.
22 Huong DL, Weschler B, Piette JC, Arfi S, Gallinary C, Frank I. Risk of ovulation-induction therapy in systemic lupus erythematosus. *Br J Rheumatol* 1996; **65**: 1184–1186.
23 Casoli P, Tumaki B, La Sala G. Fatal exacerbation of SLE after induction of ovulation. *J Rheumatol* 1997; **24**: 1640.
24 Van Vollenhoven RF. Dehydroepiandrosterone in systemic lupus erythematosus. *Rheum Dis Clin North Am* 2000; **26**: 349–362.
25 Bruce IN, Laskin CA. Sex hormones in systemic lupus erythematosus: a controversy for modern times. *J Rheumatol* 1997; **24**: 1461–1463.
26 Buyon JP, Kalunian KC, Ramsey-Goldman R *et al*. Assessing disease activity in SLE patients during pregnancy. *Lupus* 1999; **8**: 677–684.
27 Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Ann N Y Acad Sci* 1999; **876**: 131–143.
28 Masuzawa T, Miyaura C, Onoe Y, Kusano K, Nozawa S, Suda T. Estrogen deficiency stimulates B cell lymphopoiesis in mouse bone marrow. *J Clin Invest* 1994; **94**: 1097.
29 Nakayama M, Otsuka K, Sato K *et al*. Activation by estrogen of the number and function of forbidden clones in intermediate T-cell receptor cells. *Cell Immunol* 1996; **172**: 163–171.
30 Sthoeger Z, Chiorazzi N, Lahita R. Regulation of the immune response by sex hormone. *J Immunol* 1988; **141**: 91–98.
31 Kanda N, Tamaki K. Estrogen enhances immunoglobulin production by human PBMCs. *J Allergy Clin Immunol* 1999; **103**: 282–288.
32 Alcocer Varela J, Richaud Patin Y, Lorente L. High levels of TH2 cytokine gene expression in patients with systemic lupus erythematosus. Presented at the Fourth International Conference on SLE, Jerusalem, Israel. *Lupus* 1995; **4**: 4.
33 Rousset F, Garcia E, DeFrance T *et al*. Human and viral IL-10 are potent growth and differentiation factors for activated human B lymphocytes. *Proc Natl Acad Sci USA* 1991; **89**: 1890–1893.
34 Handwerger BS, Luzina I, daSilva L, Storrer CE, Via CS. Lupus: molecular and cellular pathogenesis. In Kammer GM, Tsokos GC eds. *Cytokines in the immunopathogesis of Lupus*, vol.20, Totowa, NJ: Humana Press, 1999, pp 321–340.
35 Finck BK, Chan B, Wofsy D. Interleukin-6 promotes murine lupus in NZB/NZW F1 mice. *J Clin Invest* 1994; **94**: 585–591.
36 Kalechman Y, Gafer U, Da JP, Albeck M, Alarcon-Segovia D, Sredni B. Delay in the onset of systemic lupus erythematosus following treatment with the immunomodulator AS101: association with IL-10 inhibition and increase in TNF alpha levels. *J Immunol* 1997; **159**: 2658–2667.
37 Kim HR, Ryu SY, Kim HS *et al*. Administration of dehydroepiandrosterone reverses the immune suppression induced by high doses of antigen in mice. *Immunol Invest* 1995; **24**: 583–593.
38 Dayan M, Zinger H, Kalush F *et al*. The beneficial effects of treatment with tamoxifen and anti-estradiol antibody on experimental systemic lupus erythematosus are associated with cytokine modulation. *Immunology* 1997; **90**: 101–108.
39 Angstwurm MWA, Gartner R, Loms Ziegler-Heitbrock HW. Cyclic plasma IL-6 levels during normal menstrual cycle. *Cytokine* 1997; **9**: 370–374.
40 Verthelyi D, Klinman DM. Sex hormone levels correlate with the activity of cytokine-secreting cells *in vivo*. *Immunology* 2000; **10**: 384–390.
41 Suenaga R, Mitamura K, Evans MJ, Abdou NI. Binding affinity and quantity of estrogen receptor in peripheral blood monocytes of patients with systemic lupus erythematosus. *Lupus* 1996; **5**: 227–231.
42 Dhaher YY, Greenstein B, Fougerolles Nunn E, Khamashta M, Hughes GR. Stain differences in binding properties of estrogen receptors in immature and adult BALB/c and MRL/MP-lpr/lpr mice, a model of systemic lupus erythematosus. *Int J Immunopharmacol* 2000; **22**: 247–254.
43 Denman AM. Systemic lupus erythematosus – is a viral aetiology a credible hypothesis? *J Infect* 2000; **40**: 229–233.
44 Hishikawa T, Kaneko H, Shirasawa T *et al*. Detection of antibodies to recombinant gag protein derived from human endogenous retrovirus like sequence, clone 4–1, in autoimmune diseases. *Viral Immunol* 1997; **10**: 137–147.
45 Ogasawara H, Naito T, Kaneko H *et al*. Quantitative analyses of messenger RNA of human endogenous retrovirus in SLE patients. *J Rheumatol* 2001; **28**: 533–538.

46 Naito T, Ogasawara H, Kaneko H *et al.* Immune abnormalities induced by human endogenous retroviral peptides: with reference to the pathogenesis of systemic lupus erythematosus. *J Clin Immunol* 2003; **23**: 369–374.
47 Groudine M, Eisenman R, Weintraub H. Chromatin structure of endogenous retroviral genes and activation by an inhibitor of DNA methylation. *Nature* 1981; **292**: 311–317.
48 Sekigawa I, Okada M, Ogasawara H, Kaneko H, Hishikawa T, Hashimoto H. DNA methylation in systemic lupus erythematosus. *Lupus* 2003; **12**: 79–85.
49 Ono M, Kawakami M, Ushikubo H. Stimulation of expression of the human endogenous retrovirus genome by female steroid hormones in human breast cancer cell line T47D. *J Virol* 1987; **61**: 2059–2062.
50 Van Der Burg G, Van Selm-Mitenburg AJP, De Laat SW, Van Zoelen EJJ. Direct effects of estrogen on c-fos and c-myc proto-oncogene expression and cellular proliferation in human breast cancer cells. *Mol Cell Endocrinol* 1989; **64**: 223–228.
51 Szijan I, Parma DL, Engel NI. Expression of c-myc and c-fos proto-oncogenes in the anterior pituitary gland of the rat, effect of estrogen. *Horm Metab Res* 1992; **24**: 154–157.
52 Richardson BC, Strahler JR, Pivirotto S *et al.* Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. *Arthritis Rheum* 1992; **35**: 647–662.
53 Yung R, Powers D, Johnson K *et al.* Mechanisms of drug-induced lupus. II. T cells overexpressing lymphocyte function-associated antigen 1 become autoreactive and cause a lupus-like disease in syngeneic recipients. *J Clin Invest* 1996; **97**: 2866–2871.
54 Deng C, Kaplan MJ, Yang J *et al.* Decreased ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. *Arthritis Rheum* 2001; **44**: 397–407.
55 Yung R, Ray D, Eisenbraun JK *et al.* Unexpected effects of a heterozygous dnmt1 null mutation on age-dependent DNA hypomethylation and autoimmunity. *J Gerontol A Biol Sci Med Sci* 2001; **56**: 268–276.
56 Deng C, Lu Q, Zhang Z *et al.* Hydralazine may induce autoimmunity by inhibiting extracellular signal-regulated kinase pathway signaling. *Arthritis Rheum* 2003; **48**: 746–756.
57 Kuwabara N, Kondo N, Fukutomi O, Fujii H, Orii T. Methylation pattern of I epsilon region in B cells stimulated with interleukin 4 and Epstein-Barr virus in patients with a high level of serum IgE. *Eur J Immunogenet* 1995; **22**: 265–275.
58 Kiriyama K, Sugiura H, Uehara M. Premenstrual deterioation of skin symptoms in female patient with atopic dermatitis. *Dermatology* 2003; **206**: 110–102.
59 Petri M, Allbritton J. Antibiotic allergy in systemic lupus erythematosus: a case-control study. *J Rheumatol* 1992; **19**: 265–269.
60 Elkayan O, Tamir R, Wysenbeek A. Serum IgE concentrations, disease activity, and atopic disorders in systemic lupus erythematosus. *Allergy* 1995; **50**: 94–96.
61 Morton S, Palmer B, Muir K, Powell RJ. IgE and non-IgE mediated allergic disorders in systemic lupus erythematosus. *Ann Rheum Dis* 1998; **57**: 660–663.
62 Sekigawa I, Yoshiike T, Iida N, Hashimoto H, Ogawa H. Allergic disorders in systemic lupus erythematosus: prevalence and family history. *Lupus* 2002; **11**: 426–429.
63 Sekigawa I, Yoshiike T, Iida N, Hashimoto H, Ogawa H. Two cases of atopic dermatitis associated with autoimmune abnormalities. *Rheumatology* 2003; **42**: 184–185.
64 Sekigawa I, Tokano Y, Yoshiike T, Iida N, Hashimoto H, Ogawa H. Relationship between serum IgE and autoantibody levels in SLE patients. *Clin Exp Rheumatol* 2003; **21**: 683.
65 Sekigawa I, Yamada M, Iida I, Hashimoto H, Ogawa H. Comparison of serum IgE levels between female and male SLE patients: with reference to gender differences in the incidence of SLE. *Clin Exp Rheumatol* 2004 (in press).
66 Han D, Denison MS, Tachibana H, Yamada K. Effects of estrogenic compounds on immunoglobulin production by mouse splenocytes. *Biol Pharm Bull* 2002; **25**: 1263–1267.
67 Voulgri PV, Katsimbri P, Alamanos Y, Drosos AA. Gender and age differences in systemic lupus erythematosus. A study of 489 Greek patients, with a review of the literature. *Lupus* 2002; **11**: 722–729.