AOP ID and Title:

AOP 314: Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)
Short Title: Binding to ER-α leading to exacerbation of SLE

Authors

Yasuharu Otsubo (1) Takao Ashikaga (1) Tomoki Fukuyama (1) Ken Goto (1) Shinko Hata (1) Shigeru Hisada (1) Shiho Ito (1) Hiroyuki Komatsu (1) Sumie Konishi (1) Tadashi Kosaka (1) Kiyoshi Kushima (1) Shogo Matsumura (1) Takumi Ohishi (1) Junichiro Sugimoto (1) Yasuhiro Yoshida (1)

(1) AOP Working Group, Testing Methodology Committee, The Japanese Society of Immunotoxicology

Corresponding author: Yasuharu Otsubo (otsubo-yasuharu@snbl.co.jp)

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite	Under Development	1.73	Included in OECD Work Plan

Abstract

This AOP describes the linkage between the binding to estrogen receptor (ER) α in immune cells with the exacerbation of the autoimmune disease systemic lupus erythematosus (SLE).

Estrogen receptors (ERs), ERα and ERβ, are a group of proteins that are activated by the steroid hormone estrogen and are widely expressed in most tissue types, including most immune cells. ERα can be activated with exogenous and endogenous estrogens. Also, there are numerous xenoestrogens that exist in the environment and imitate estrogen. Bisphenol A (BPA) is an example of a xenoestrogen that is considered an endocrine disrupting (ED) compound. SLE is an autoimmune disease characterized by overproduction of a variety of anti-cell nuclear and other pathogenic autoantibodies. It is characterized by B-cell hyperactivity, polyclonal hypergammaglobulinemia, and immune complex deposition.

Binding to ERα in immune cells by a xenoestrogen or endogenous estrogen marks the molecular initiating event (MIE), which results in induction of GATA3 expression (KE1). One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper2 (Th2) activity, however GATA3 expression induce increase of Th2 cells producing cytokine interleukin-4 (IL-4) (KE2), which results in increase of anti-DNA antibody from autoreactive B cell (KE3). This sequence of pathway means that the immune system skew from a Th1 to a Th2 profile, which results in the adverse outcome (AO) of exacerbated SLE.

We have identified a number of key events along this pathway and determined the key event relationships, based on which we have created an AOP for binding to ERα in immune cells leading to exacerbated SLE.

Background

It is well recognized that allergic diseases and autoimmune diseases are markedly increased the last several decades. About the same time, increasing scientific and social attention had been paid to environmentally dispersed chemicals that can enter the body by ingestion or adsorption and that mimic the actions of estrogens. These chemicals are termed endocrine disruptors (EDs) or environmental estrogens and are found in plastics (bisphenol-A, phthalates), pesticides (DDT, hexachlorobenzene, and dieldrin) and the like. Some of these estrogenic chemicals have also been shown to influence the immune system. Endocrine disruptors mimic hormones, block or alter hormone binding to receptors, or alter the metabolism of natural estrogens. It has been widely noted that females have stronger immune capabilities than males, as evidenced by their better immune responses to a variety of self-antigens and non-self-antigens, or vaccination. Paradoxically, the stronger immune response comes at a steep price, which is the high incidence of autoimmune diseases in females. This phenomenon of gender-based immune capability is largely attributed to the effects of sex hormones. Estrogens regulate the level of serum and uterine IgM, IgA, and IgG, and they augment antibody production to several nonself- antigens and self-antigens. It is possible that endocrine disruptors that mimic estrogenic activity may be involved in the increased incidence of autoimmune diseases such as SLE (Yurino H. 2004, Vaishali RM. 2018).

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	1710	Binding to estrogen receptor (ER)-α in immune cells	Binding to estrogen receptor (ER)-α

Sequence	Type	Event ID	Title	Short name
	KE	1711	Induction of GATA3 expression	Induction of GATA3 expression
	KE	1712	Increase of Th2 cells producing IL-4	Increase of Th2 cells producing IL-4
	KE	1713	Increase of anti-DNA antibody from autoreactive B cell	Increase of autoantibody production
	AO	1714	Exacerbation of systemic lupus erythematosus (SLE)	Exacerbation of SLE

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Binding to estrogen receptor (ER)-α in immune cells	adjacent	Induction of GATA3 expression	Moderate	Moderate
Induction of GATA3 expression	adjacent	Increase of Th2 cells producing IL-4	Moderate	Moderate
Increase of Th2 cells producing IL-4	adjacent	Increase of anti-DNA antibody from autoreactive B cell	Moderate	Moderate
Increase of anti-DNA antibody from autoreactive B cell	adjacent	Exacerbation of systemic lupus erythematosus (SLE)	Moderate	Moderate

Stressors

Name	Evidence
Bisphenol A	Moderate
17beta-Estradiol	High

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI

Sex Applicability

Sex	Evidence
Mixed	High

It has long been appreciated that most autoimmune disorders are characterized by increased prevalence in females, suggesting a potential role for sex hormones (estrogen) in the etiology of autoimmunity. Females generally exhibit a stronger response to a variety of antigens including ERα ligands than males, which is perhaps one reason that they are more prone to develop autoimmune and allergic diseases such as SLE in greater severity than males. Therefore, this AOP is applicable to females and is dependent on the levels of estrogen, which means it varies with life stage, and age.

SLE frequently develop and progress in setting in which sympathoadrenomedullary and gonadal hormone levels are changing, e.g., during pregnancy, the postpartum period, or estrogen administration in menopause (Wilder RL. 1999). Women using oral contraceptives that contain estrogen or undergoing hormone replacement therapy are susceptible to major flare ups and exacerbation of the disease (Whitelaw DA. 2007).

The mechanisms described in this AOP are applicable to rodents and humans, and then the findings of this AOP are not found in any other species. However, Th2 dominant conditions induced by binding to ERα is considered likely to occur in a variety of mammalian species since ERα are expressed in all vertebrates (Eick GN. 2011).

Essentiality of the Key Events

Stressor , MIE and later events:

The NZB/W F1 mouse is the oldest classical model of lupus generated by the F1 hybrid between the NZB and NZW strains. The administration of the estrogen antagonist tamoxifen diminishes immune complex deposition in the kidneys and increases survival in NZB/W F1 strain. Renal disease was evaluated by the development of albuminuria and histological changes in the kidney (Wu WM. 2000). In females of the NZB/NZW F1 strain, disruption of ERα attenuated glomerulonephritis and increased survival and reduced anti-dsDNA antibodies (Bynote KK. 2008, Isenberg DA. 2007) and ovariectomy of NZB/W F1 mice not only delayed onset of the disease but also decreased autoantibody titer Meanwhile, restoration of estradiol in ovariectomized NZB/W F1 mice reestablished high numbers of autoantibody-producing (DNA-specific) B cells, and thereby suggests a pathogenic role of estrogen in lupus (Daniel P. 2011). Both NZB and NZW display limited autoimmunity, while NZB/W F1 hybrids develop severe lupus-like phenotypes comparable to that of lupus patients. In NZM female mice, ERα inactivation markedly prolonged life-span, lowered proteinuria, and ameliorated glomerulonephritis but resulted in higher serum anti-dsDNA antibody levels (Svenson JL. 2008).

KE1 and later events:

GATA3 mRNA expression has potential to induced IL-4 production in CD4+T cell (Lambert KC. 2005). The differentiation of activated CD4+T cells into the T helper type 1 (Th1) or Th2 fate is regulated by cytokines and the transcription factors T-bet and GATA-3. Early GATA-3 expression, required for Th2 differentiation, was induced by T cell factor 1 (TCF-1) and its cofactor β-catenin, mainly from the proximal Gata3 promoter upstream of exon 1b. TCF-1 blocked Th1 fate by negatively regulating interferon-γ (IFN-γ) expression independently of β-catenin. Thus, TCF-1 initiates Th2 differentiation of activated CD4+T cells by promoting GATA-3 expression and suppressing IFN-γ expression. Higher GATA-3 expression promotes IL-4 production and initiates Th2 differentiation (Qing Y. 2009). GATA-3 mRNA expression also increased in patients with SLE, compared with the healthy control groups (Zheng H. 2015, Sonia GR. 2012).

KE2 and later events:

Administration of mAb against IL-4 before the onset of lupus was effective in preventing the onset of lupus nephritis (Nakajima A. 1997).

KE3 and later events:

In a study to investigate a novel subpopulation of B-1 cells and its roles in murine lupus, anti-double-stranded DNA (anti-dsDNA) autoantibodies were preferentially secreted by a subpopulation of CD5+ B-1 cells that expressed programmed death ligand 2 (L2pB1 cells) (Xuemei Z. 2009). A substantial proportion of hybridoma clones generated from L2pB1 cells reacted to dsDNA. L2pB1 cells are potent antigen-presenting cells and a dramatic increase of circulating L2pB1 cells in lupus-prone BXSB mice correlates with elevated serum titers of anti-dsDNA antibodies (Xuemei Z. 2009).

Weight of Evidence Summary

Biological Plausibility

KER	KE_{up}-KE_{down}	Plausibility	Rationales supported by literatures
KER 1	Binding, Estrogen receptor α in immune cells - Induction, GATA3 expression	Weak	In immune cells, this event is confirmed indirectly; using artificial STAT6-ER fusion protein.
KER 2	Induction, GATA3 expression - Increase, Th2 cells producing IL-4	Strong	XXXX
KER 3	Increase, Th2 cells producing IL-4 - Increase, anti-DNA antibody production from autoreactive B cell	Weak	XXXX
KER 4	Increase, anti-DNA antibody production from autoreactive B cell -	Strong	XXXX

Empirical Support

KER	Empirical support of KERs
MIE=>KE 1 Binding, Estrogen receptor α in immune cells leads to Induction, GATA3 expression	Empirical support of the MIE => KE1 is weak. Rationale

	MIE: XXX KE XX: XXXX
KE 1=> KE 2: Induction, GATA3 expression leads to Increase, Th2 cells producing IL-4	Empirical support of the KE 1=> KE 2 is strong. Rationale KE XX: XXXX AO: XXXX
KE 2=> KE 3: Increase, Th2 cells producing IL-4 leads to Increase, anti-DNA antibody production from autoreactive B cell	Empirical support of the KE 2=> KE 3 is weak. Rationale KE XX: XXXX AO: XXXX
KE 3=>AO: Increase, antibody production from anti-DNA antibody production from autoreactive B cell leads to Exacerbation, systemic lupus erythematosus (SLE)	Empirical support of the KE 3 => AO is strong. Rationale KE XX: XXXX AO: XXXX

Quantitative Consideration

KER1

$CD4^+$T cell expressed GATA3 mRNA cultured with 10^{-9} M (272.4 pg/mL) concentrations of 17β-estradiol for 12-16 hr (Lambert KC. 2005).

BPA (0.1 mM) also indirectly induced GATA3 expression of Th cells, and this effect is mediated by dendritic cells exposed to BPA for 24 hr (Guo H. 2010). Naïve Th cells increased GATA3 expression cultured with dendritic cells exposure of BPA (0.1 mM) for 7 days.

KER2

Pre-stimulation 16 hr of 17β-estradiol (the concentration 10^{-9} M = 272.4 pg/mL) increased IL-4 secretion from $CD4^+$T cell (Lambert KC. 2005).

KER3

PBMCs or B cells were cultured for 7 days with 17β-estradiol (10^{-8} mol/L) and then, IgG and IgM production were increased up to about 150% (PBMC) and 200% (B cells) (Kanda N. 1999).

KER4

XXXX

References

1. Yurino, H., Ishikawa, S., Sato, T., Akadegawa, K., Ito, T., Ueha, S., Inadera, H. and Matsushima, K. (2004). Endocrine disruptors (environmental estrogens) enhance autoantibody production by B1 cells. Toxicological Sciences 81(1): 139-147.
2. Vaishali RM. Sex Hormones in Acquired Immunity and Autoimmune Disease. Frontiers in Immunology 2018. 9: 2279; 1-21.
3. Wilder RL, Elenkov IJ, Hormonal regulation of tumor necrosis factor-alpha, interleukin-12 and interleukin-10 production by activated macrophages. A disease-modifying mechanism in rheumatoid arthritis and systemic lupus erythematosus? Ann N Y

Acad Sci. 1999. 22; 876:14-31.
4. Whitelaw DA, Jessop SJ. Major flares in women with SLE on combined oral contraception. Clin Rheumatol. 2007; 26(12):2163-2165.
5. Eick GN, Thornton JW. Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. Molecular and cellular endocrinology. 2011; 334: 31-38.
6. Wu WM, Lin BF, Su YC, et al. (2000). Tamoxifen decreases renal inflammation and alleviates disease severity in autoimmune NZB/W F1 mice. Scandinavian Journal of Immunology 52(4): 393-400.
7. Bynote, KK, Hackenberg, JM., Korach, K.S., Lubahn, D. B., Lane, P. H. and Gould, K. A. (2008). Estrogen receptor-alpha deficiency attenuates autoimmune disease in (NZB xNZW) F1 mice. Genes and Immunity. 9: 137-152.
8. Isenberg, DA., Manson, JJ., Ehrenstein, MR. and Rahman, A. (2007). Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology 46:1052-6.
9. Daniel, P., Allison, S., Yiming, Y., Ying-Yi, Z. and Laurence, M. Murine Models of Systemic Lupus erythematosus. Journal of Biomedicine and Biotechnology 2011: ArticleID 271694
10. Svenson JL, EuDaly J, Ruiz P, Korach KS, Gilkeson GS. Impact of estrogen receptor deficiency on disease expression in the NZM2410 lupus prone mouse. Clin Immunol. 2008;128(2):259-68.
11. Lambert KC, Curran EM, et al. Estrogen receptor alpha (ERalpha) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17beta-estradiol acts through ERalpha to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol. 2005; 175(9): 5716-23.
12. Qing Y., Archna S., Sun Y. O., Hyung-Geun M., M Zulfiquer H., Theresa M. S., Karen E. L., Hansen D., Beibei W., Marian L. W., Zhou Z. and Jyoti M. S., T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-γ. Nat Immunol. 2009; 10(9): 992–999.
13. Zheng H, Guo X, Zhu Y, et al., Distinct role of Tim-3 in systemic lupus erythematosus and clear cell renal cell carcinoma. Int J Clin Exp Med 2015;8(5):7029-7038.
14. Sonia GR, et al. Altered AKT1 and MAPK1 Gene Expression on Peripheral Blood Mononuclear Cells and Correlation with T-Helper-Transcription Factors in Systemic Lupus Erythematosus Patients. Mediators of Inflammation 2012, Article ID 495934
15. Nakajima A, Hirose S, Yagita H and Okumura K, Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. J Immunol 1997; 158 (3) 1466-1472.
16. Xuemei, Z., Stanley, L., et al. (2009). A Novel Subpopulation of B-1 Cells Is Enriched with Autoreactivity in Normal and Lupus-Prone Mice. Arthritis & Rheumatology 60 (12):3734-3743.
17. Guo H, Liu T, Ling F, et al. Bisphenol A in combination with TNF-alpha selectively induces Th2 cell-promoting dendritic cells in vitro with an estrogen-like activity. Cell Mol Immunol. 2010;7(3):227-34.
18. Kanda N. and Tamaki, K. (1999). Estrogen enhances immunoglobulin production by human PBMCs. The Journal of Allergy and Clinical Immunology 103(2): 282-288.

Appendix 1

List of MIEs in this AOP

Event: 1710: Binding to estrogen receptor (ER)-α in immune cells

Short Name: Binding to estrogen receptor (ER)-α

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:314 - Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	MolecularInitiatingEvent

Stressors

Name
Bisphenol A
17beta-Estradiol
Propylpyrazoletriol

Biological Context

Level of Biological Organization
Molecular

Organ term

Organ term
immune system

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

E_2 activates ERα and ERβ with the same affinity although they share only 56% similarity in their ligand binding domains (Monroe DG. 2005, Papoutsi Z. 2009). Exposure E_2 induced thymic atrophy, and changing T-cell phenotype (decreasing double positive ($CD4^+CD8^+$) T cell and increasing double negative ($CD4^-CD8^-$) T cell) in thymus (Okasha SA. 2001).

BPA binds to both ERα and ERβ, and ERα binding affinity of BPA is lower than that of ERβ (Takayanagi S. 2006). While these bindings are less than 2000-fold affinity compared to the binding of estradiol to estrogen receptors (Krishnan AV. 1993).

Propylpyrazoletriol (PPT) is an ERα-selective agonist, which shows 410-fold selectivity for ERα as compared with ERβ (Kraichely DM. 2000, Li J. 2006). Li et al (2006) demonstrated that ovariectomized mice exposed PPT induced severe thymic atrophy, changing T-cell phenotype (CD4/CD8 phenotype profile) in thymus, and a reduction of mature B cell number in spleen. Since these effects by PPT were equal to or greater than E2, ERα plays the predominant role in the upregulation of immune responses.

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI
Mus musculus	Mus musculus	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	

Since ERα expresses in the cells of a vast variety of (vertebrate) species (Maria B. 2015) and there is common functionality in the immune systems of at least humans and mice, this AOP might be applicable to many mammal species, including humans and rodents. The estrogen receptors are composed of several domains important for hormone binding, DNA binding, dimer formation, and activation of transcription (Green S. 1986, Kumar V. 1986, Warnmark A. 2003). Interspecies sequence identities for the entire ERα are 88.5% (human-mouse), 87.5% (human-rat), and 97.5% (mouse-rat). For the ligand binding domain (ERα-LBD) alone, the interspecies sequence identities are 95.5% (human-mouse), 95.1% (human-rat), and 99.2% (mouse-rat) (White R. 1987).

ERα is widely expressed in most tissue types including most immune cells in males and females (Couse JF. 1997, Chelsea C. 2017). The ERs' expression patterns and functions vary in a receptor subtype, cell- and tissue-specific manner. In the adult human, large-scale sequencing approaches show that ERα mRNA is detected in numerous human tissues, with the highest levels in the uterus, liver, ovary, muscle, mammary gland, pituitary gland, adrenal gland, spleen and heart, and at lower levels in the prostate, testis, adipose tissue, thyroid gland, lymph nodes and spleen (Fagerberg L. 2014, Sayers EW. 2012) (www.ncbi.nlm.nih.gov/UniGene).

Estrogen level is higher in women than men. Ordinary estrogen levels in women are 20-30 pg/mL during diestrus, 100-200 pg/mL during estrus, and 5000-10000 pg/mL during pregnancy (Offner H. 2000). Therefore, the influence of ligand binding to ERα in immune cells is expressed more strong in women than men, especially high estrogen level period.

Key Event Description

ERα is expressed in all vertebrates (Eick GN. 2011). ERα was discovered in the late 1960s and was cloned and characterized in 1985 (Melissa C. 2011). ERα is expressed in a variety of immunocompetent cells, including thymocytes, CD4+ (Th1, Th2, Th17, and Tregs) and CD8+ cells and macrophages (Melissa C. 2011, Salem ML. 2004, Robinson DP. 2014). One study examined ERα expression in resting and activated PBMC subsets and found that ERα was expressed at higher levels in thymocytes, CD4+ T cells than B cells (Melissa C. 2011). ERα is a nuclear hormone transcription factor that classically binds with ligand (stressors), further stabilizing dimers that subsequently bind estrogen response elements (ERE) or non-ERE to transactivate or suppress specific target

genes (Parker MG. 1993, Goldstein RA. 1993, Sasson S. 1991, Brandt ME. 1997, Carolyn MK. 2001).

How it is Measured or Detected

The binding affinities of E_2 and BPA for ERα can be confirmed by radio receptor assay, and its dimer dissociation is measured using size exclusion chromatography (Brandt ME. 1997, Takayanagi S. 2006, OECD TG440 [*in vivo*] and TG455 [*in vitro*]). While the binding affinities of PPT for ERα was determined by competitive radiometric binding assays by chemiluminescence (Kraichely DM. 2000, Carlson KE. 1997).

References

1. Eick GN, Thornton JW. Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. Molecular and cellular endocrinology. 2011; 334: 31-38.
2. Melissa, C. and Gary, G (2011). Estrogen Receptors in Immunity and Autoimmunity. Clinical Reviews in Allergy & Immunology 40:66-73.
3. Salem ML. (2004). Estrogen, a double-edged sword: modulation of Th1- and Th2-mediated inflammations by differential regulation of Th1/Th2 cytokine production. Current Drug Targets - Inflammation & Allergy 3(1): 97-104.
4. Robinson DP, Hall, O. J., Nilles, T. L., Bream, J.H. and Klein, S.L. (2014). 17β-estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. Journal of Virology 88 (9): 4711-4720.
5. Parker MG, Arbuckle N, Dauvois S, Danielian P, White R. Structure and function of the estrogen receptor. Ann N Y Acad Sci. 1993. 684:119-26.
6. Goldstein RA, Katzenellenbogen JA, Wolynes PG, et al. Three-dimensional model for the hormone binding domains of steroid receptors. Proc Natl Acad Sci. 1993;90 (21):9949-53.
7. Sasson S. Equilibrium binding analysis of estrogen agonists and antagonists: relation to the activation of the estrogen receptor. Pathol Biol (Paris). 1991;39(1):59-69.
8. Brandt ME, Vickery LE. Cooperativity and dimerization of recombinant human estrogen receptor hormone-binding domain. J Biol Chem. 1997;272(8):4843-9.
9. Carolyn MK. Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res. 2001 Jul 15; 29(14): 2905-2919.
10. Takayanagi, S. Tokunaga, T., et al. (2006). Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor γ (ERRγ) with high constitutive activity. Toxicology Letters, 167 (2):95-105.
11. OECD Guideline for the Testing of Chemicals [Test No. 440: Uterotrophic Bioassay in Rodents]
12. OECD Guideline for the Testing of Chemicals [Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists]
13. Kraichely, DM. Sun, J. Katzenellenbogen, JA. Katzenellenbogen, BS. (2000). Conformational changes and coactivator recruitment by novel ligands for estrogen receptor-α and estrogen receptor-β: correlations with biological character and distinct differences among SRC coactivator family members. Endocrinology, 141 (10):3534–3545.
14. Carlson, KE. Choli, I. Gee, A. Katzenellenbogen, BS. Katzenellenbogen, JA. (1997) Altered Ligand Binding Properties and Enhanced Stability of a Constitutively Active Estrogen Receptor: Evidence That an Open Pocket Conformation Is Required for Ligand Interaction. Biochemistry, 36:14897-14905.
15. Maria, B., Ruixin, H., Chin-Yo, L., Cecilia, W., Jan-Ake, G. (2015). Estrogen receptor signaling during vertebrate development. Biochim Biophys Acta 1849: 142-151.
16. Green S, Walter P, Chambon P, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature. 1986; 320:134-139.
17. Kumar V, Green S, Chambon P, et al. Localisation of the oestradiol-binding and putative DNA-binding domains of the human oestrogen receptor. The EMBO journal. 1986; 5: 2231-2236.
18. Warnmark A, Treuter E, Gustafsson JA, et al. Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Molecular endocrinology (Baltimore, Md). 2003; 17:1901-1909.
19. White, R., Lees, JA., Needham, M., Ham, J. and Parker, M. (1987). Structural Organization and Expression of the Mouse Estrogen Receptor. Molecular Endocrinology 1 (10): 735-744.
20. Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS. (1997) Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalphaknockout mouse. Endocrinology 138(11):4613-4621.
21. Fagerberg L, Hallstrom BM, Edlund K, et al. Analysis of the human tissue- specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Molecular & cellular proteomics. 2014; 13:397-406.
22. Sayers EW, Barrett T, Federhen S, et al. Database resources of the National Center for Biotechnology Information. Nucleic acids research. 2012; 40: D13-25.
23. Offner H, Adlard K, Zamora A, Vandenbark AA. Estrogen potentiates treatment with T-cell receptor protein of female mice with experimental encephalomyelitis. J Clin Invest. 2000;105(10):1465-72.
24. Monroe DG, Secreto FJ, Subramaniam M, Getz BJ, Khosla S, Spelsberg TC. Estrogen receptor alpha and beta heterodimers exert unique effects on estrogen- and tamoxifen-dependent gene expression in human U2OS osteosarcoma cells. Molecular endocrinology (Baltimore, Md). 2005; 19:1555–1568.
25. Papoutsi Z, Zhao C, Putnik M, Gustafsson JA, Dahlman-Wright K. Binding of estrogen receptor alpha/beta heterodimers to chromatin in MCF-7 cells. J Mol Endocrinol. 2009; 43:65-72.
26. Okasha SA, Ryu S, Do Y, McKallip RJ, Nagarkatti M, Nagarkatti PS. Evidence for estradiol-induced apoptosis and dysregulated T cell maturation in the thymus. Toxicology. 2001, 163 (1):49-62.
27. Takayanagi S, Tokunaga T, Liu X, Okada H, Matsushima A, Shimohigashi Y. Endocrine disruptor bisphenol A strongly binds

to human estrogen-related receptor γ (ERRγ) with high constitutive activity. Toxicology Letters, 2006, 167 (2):95-105.
28. Krishnan, AV., Stathis, P., Permuth, S. F., Tokes, L. and Feldman, D. (1993). Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132; 2279-2286.
29. Li, J., McMurray, RW. (2006). Effects of estrogen receptor subtype-selective agonists on immune functions in ovariectomized mice. International Immunopharmacology, 6 (9):1413-1423.

List of Key Events in the AOP

Event: 1711: Induction of GATA3 expression

Short Name: Induction of GATA3 expression

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:314 - Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	KeyEvent

Stressors

Name
17beta-Estradiol
Bisphenol A
4-Hydroxytamoxifen

Biological Context

Level of Biological Organization
Cellular

Organ term

Organ term
immune system

Evidence for Perturbation by Stressor

17beta-Estradiol

Expression of GATA3 was induced in $CD4^+$T cells treated with E_2 at a concentration of 10^{-9} M (272.4 pg/mL) for 12-16 hours (Lambert KC. 2005). GATA3 expression has potential to induced IL-4 production in $CD4^+$T cell. In contrast, expression of T-bet was decreased, which means E_2 skew the immune system from a Th1 to a Th2 profile (Lambert KC. 2005).

Bisphenol A

GATA3 expression is induced in Th cells primed by dendritic cells exposed to BPA (Guo H. 2010). Purified naive T cells were cultured and expanded under Th1 culture conditions in the presence or absence of 0.3 µM 4-HT (Research Biochemicals Institute) for 2 weeks starting from days 1, 7, 14, or 21 (Kurata H. 1999).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence

All life stages

Life Stage	Evidence

Sex Applicability

Sex	Evidence
Mixed	

Involvement of GATA3 in Th2 cell development through ER is common in humans, rodents, and other mammalian species (Ho IC. 2009). protein sequence conservation between all six vertebrate members (mouse, human, dog, cow, armadillo, capuchin and opossum) identifies GATA3 as having the highest sequence similarity with both its GATA paralogs and orthologs, suggesting that it may be closest to the ancestral mammalian GATA factor (Tremblay M. 2018).

Key Event Description

Naïve CD4 T cells can differentiate into several different types of T helpers, and Th2 cells, capable of producing IL-4, IL-5 and IL-13, are involved in humoral immunity against extracellular pathogens and in the induction of asthma and other allergic diseases. It was reported that GATA-3 promotes Th2 responses through three different mechanisms (Zhu J. 2006). Cell fate determination in each lineage requires at least two types of transcription factors: the master regulators (GATA3) as well as the signal transducers and activator of transcription (STAT) proteins (Zhu J. 2010). A direct role in bridging distant regulatory elements has been demonstrated for GATA3 at Th2 cytokine loci (Spilianakis and Flavell, 2004). GATA3 is the Th2 master regulator (Zhu J 2010, Sung-Yun. 2004, Zhu J. 2004, Zheng W. 1997, Zhang DH. 1997), but it also plays important roles in multiple steps of CD4 T cell development (Ho IC. 2009). GATA3 can act as pioneer factors by initiating local chromatin opening and allowing the recruitment of other transcription factors to regulatory elements (Spilianakis and Flavell, 2004). Th2 differentiation is completely abolished both in vitro and in vivo when GATA3 is conditionally deleted in peripheral CD4 T cells (Zhu J. 2004, Pai SY. 2004). GATA-3 mRNA expression also increased in patients with SLE, compared with the healthy control groups (Zheng H. 2015, Sonia GR. 2012).

How it is Measured or Detected

GATA3 mRNA in CD4 T cells can be detected by Real-time PCR (RT-PCR) (Lambert KC. 2005, Kurata H. 1999, Zhu J. 2001).

References

1. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol. 2010; 28:445-89.
2. Spilianakis CG & Flavell RA, Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. Nature Immunology. 2004; 5: 1017-1027.
3. Zhu J, Paul WE. Peripheral CD4 T cell differentiation regulated by networks of cytokines and transcription factors. Immunol Rev. 2010; 238(1):247-62.
4. Sung-Yun, Morgan L. T. I-Cheng H. (2004). GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proceedings of the National Academy of Sciences. 101 (7): 1993-1998.
5. Zhu J, Min B, Paul WE, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5(11):1157-65.
6. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997. 16;89(4):587-96.
7. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997. 22;272(34):21597-603.
8. Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: essential functions before and after Thelper-2-cell differentiation. Nat Rev Immunol. 2009;9(2):125-35.
9. Zheng H, Guo X, Zhu Y, et al., Distinct role of Tim-3 in systemic lupus erythematosus and clear cell renal cell carcinoma. Int J Clin Exp Med 2015;8(5):7029-7038.
10. Sonia GR, et al. Altered AKT1 and MAPK1 Gene Expression on Peripheral Blood Mononuclear Cells and Correlation with T-Helper-Transcription Factors in Systemic Lupus Erythematosus Patients. Mediators of Inflammation 2012, Article ID 495934
11. Lambert KC, Curran EM, et al. Estrogen receptor alpha (ERalpha) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17beta-estradiol acts through ERalpha to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol. 2005; 175(9): 5716-23.
12. Kurata, H., Lee, H. J., O'Garra, A. and Arai, N. (1999). Ectopic expression of activated STAT6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 11: 677-688.
13. Zhu, J., Guo, L., Watson, C. J., Hu-Li, J. and Paul, W. E. (2001). STAT6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. The Journal of Immunology 166(12): 7276-7281.
14. Tremblay M, GATA transcription factors in development and disease. 2018; 22:145(20).
15. Guo H, Liu T, Ling F, et al. Bisphenol A in combination with TNF-alpha selectively induces Th2 cell-promoting dendritic cells in vitro with an estrogen-like activity. Cell Mol Immunol. 2010;7(3):227-34.

Event: 1712: Increase of Th2 cells producing IL-4

Short Name: Increase of Th2 cells producing IL-4

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:314 - Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	KeyEvent

Stressors

Name
17beta-Estradiol
Bisphenol A

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
T-helper 2 cell

Organ term

Organ term
immune system

Evidence for Perturbation by Stressor

17beta-Estradiol

In vitro, the addition of E2 significantly increased IL-4 secretion from ERα-replete CD4+T cells, while this effect was abrogated in ERα-deficient CD4+T cells. (Lambert KC. 2005).

Bisphenol A

Mouse lymphocytes stimulated with a massive amount of BPA (50 µM) were Th2 polarized, with prominent elevation of IL-4 as well as IL-10 (Lee J. 2010). Similarly, BPA enhanced IL-4 production in antigen-activated T cells by ELISA or RT-PCR, although the concentrations of BPA that they utilized (10-50 µM) were high (Lee MH. 2003). In this experiment, IL-4 level is confirmed baseline when treated with anti-CD4 mAb. Exposure to BPA in adulthood mice promoted antigen-stimulated levels of IL-4, IL-10, and IL-13, but not IFN-γ (Huimin Y. 2008).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Production of IL-4 from Th2 is common in humans, rodents, and other mammalian species.

Key Event Description

In naive CD4+ T cells, T cell expansion shifts toward a Th2 phenotype that produces Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13, thereby increasing antibody production from autoantibody-producing B cells. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, meanwhile Th1 cells produce IL-12, TNF-α, and IFN-γ. During Th2 polarization, IL-4 produced by Th2 cell. IL-12 plays a central role in promoting the differentiation of naive CD4+ T cells into mature Th1 effector cells. Secretion of IL-10 from Th2 has been suggested to downregulate the DC-derived IL-12 production and lead to a Th2 differentiation (Aste-Amezaga M. 1998). Th2 cells produce IL-4, which stimulates B-cells to proliferate, to switch immunoglobulin classes, and to differentiate into plasma and memory cells. The receptor for IL-4 is IL-4Rα, which expresses in B cells. IL-4 also plays an important role in the development of certain immune disorders, particularly allergies and some autoimmune diseases and especially when there is Th2 polarization. Th2 cells from GATA3 and STAT6 knockout animals showed reduction in IL-4 production (Zhu J. 2004, Pai SY. 2004).

How it is Measured or Detected

The levels of IL-4 in the cell supernatants were determined by a sandwich enzyme-linked immunosorbent assay (ELISA), cytometric bead array (CBA) kits, or immunoblot analysis (Lee MH. 2003, Huimin Y. 2008, Lee J. 2010), and mRNA levels of IL-4 in the cells were assayed by reverse transcription–polymerase chain reaction (RT-PCR) (Lee MH. 2003, Lee J. 2010).

References

1. Aste-Amezaga M, Ma X, Sartori A, Trinchieri G. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. J Immunol. 1998. 15;160(12):5936-44.
2. Zhu J, Min B, Paul WE, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5(11):1157-65.
3. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc Natl Acad Sci U S A. 2004 Feb 17;101(7):1993-8.
4. Lee, MH, Chung, S. W., Kang, B. Y., Park, J., Lee, C. H., Hwang, S. Y. and Kim, T. S. (2003). Enhanced interleukin-4 production in CD4+ T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+. Immunology 109(1): 76-86.
5. Huimin, Y., Masaya, T. and Kazuo, S. (2008). Exposure to Bisphenol A Prenatally or in Adulthood Promotes Th2 Cytokine Production Associated with Reduction of CD4+CD25+ Regulatory T Cells. Environmental Health Perspective 116(4): 514–519.
6. Lee, J. and Lim K. T. (2010). Plant-originated glycoprotein (36kDa) suppresses interleukin-4 and -10 in bisphenol A-stimulated primary cultured mouse lymphocytes. Drug and Chemical Toxicology. 33(4): 421-429.
7. Lambert KC, Curran EM, et al. Estrogen receptor alpha (ERalpha) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17beta-estradiol acts through ERalpha to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol. 2005; 175(9): 5716-23.

Event: 1713: Increase of anti-DNA antibody from autoreactive B cell

Short Name: Increase of autoantibody production

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:314 - Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	KeyEvent

Stressors

Name
17beta-Estradiol
Bisphenol A
Diethylstilbestrol

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term

B cell

Organ term

Organ term

immune system

Evidence for Perturbation by Stressor

17beta-Estradiol

BPA as well as E_2 and diethylstilbestrol (DES) enhanced anti-Br-RBC autoantibody production by B1 cells in vivo. IgM production by B1 cells in the presence of ED was more prominent on aged BWF1 mice developing lupus nephritis. (Yurino H. 2004).

To examine a direct effect of endocrine disruptors on IgM antibody production by B1 or B2 cells, B1 cells were prepared from peritoneal cells and B2 cells from spleen, B1 or B2 cells were cultured in the presence of endocrine disruptors (E_2: 100 nM, DES: 100 nM, BPA: 1 µM) for 4 days (Yurino H. 2004).

Direct exposure of PBMCs from SLE patients to E_2 induces secretion of anti-dsDNA antibodies and enhances the secretion of Igs, in particular IgG (Kanda N. 1999).

In both (NZB×NZW) F1 and MRL/lpr mice, estrogen treatment exacerbates the lupus disease, with augmented levels of autoantibodies against dsDNA and phospholipids as well as formation of circulating immune complexes (Grimaldi CM. 2002, Peeva E. 2000).

Hybridomas generated from E_2-treated mice express high-affinity, unmutated anti-DNA antibodies, indicating that naı¨ve B cells that are normally deleted or anergized are rescued from tolerance induction (Bynoe MS. 2000). E_2 treatment resulted in a rise in anti-DNA serum titers and in Ig deposition in renal glomeruli (Bynoe MS. 2000).

Bisphenol A

BPA as well as E_2 and diethylstilbestrol (DES) enhanced anti-Br-RBC autoantibody production by B1 cells in vivo. IgM production by B1 cells in the presence of ED was more prominent on aged BWF1 mice developing lupus nephritis. (Yurino H. 2004).

In a murine model of SLE, BPA increased the number of B cells producing autoantibodies, and IgM antibody secretion by B1 cells was augmented (Yurino H. 2004).

To examine a direct effect of endocrine disruptors on IgM antibody production by B1 or B2 cells, B1 cells were prepared from peritoneal cells and B2 cells from spleen, B1 or B2 cells were cultured in the presence of endocrine disruptors (E_2: 100 nM, DES: 100 nM, BPA: 1 µM) for 4 days (Yurino H. 2004).

Diethylstilbestrol

BPA as well as E_2 and diethylstilbestrol (DES) enhanced anti-Br-RBC autoantibody production by B1 cells in vivo. IgM production by B1 cells in the presence of ED was more prominent on aged BWF1 mice developing lupus nephritis. (Yurino H. 2004).

To examine a direct effect of endocrine disruptors on IgM antibody production by B1 or B2 cells, B1 cells were prepared from peritoneal cells and B2 cells from spleen, B1 or B2 cells were cultured in the presence of endocrine disruptors (E_2: 100 nM, DES: 100 nM, BPA: 1 µM) for 4 days (Yurino H. 2004).

In both (NZB×NZW) F1 and MRL/lpr mice, estrogen treatment exacerbates the lupus disease, with augmented levels of autoantibodies against dsDNA and phospholipids as well as formation of circulating immune complexes (Grimaldi CM. 2002, Peeva E. 2000).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence

All life stages

Life Stage	Evidence

Sex Applicability

Sex	Evidence
Mixed	

Antibody production from B cells is common in humans, rodents, and other mammalian species. Since almost experiment are performed in female, it is considered that this event in SLE are noted more frequently in females.

Key Event Description

The receptor for IL-4 is IL-4Rα, which expresses in B cells. IL-4 produced by Th2 stimulates B-cells to proliferate, to switch immunoglobulin classes, and to differentiate into plasma and memory cells. Anti-DNA antibodies are produced from autoreactive B cell. In murine models, addition of estrogen or prolactin can lead to an autoimmune phenotype with an increase in mature high-affinity autoreactive B cells (Daniel P. 2011).

How it is Measured or Detected

[*in vivo* assay]

NZB/W F1 mice are used as model of SLE (Wu WM. 2000). BALB/c R4Ag-gamma 2b transgenic mice are used for evaluation of autoreactive B cells (Peeva E. 2005). These mice are administrated of the estrogen antagonist tamoxifen. Disruption of ERα (Bynote KK. 2008, Isenberg DA. 2007) and ovariectomy of NZB/W F1 mice are used as model of estrogen dysfunction (Daniel P. 2011). Survival and glomerulonephritis of these animals were evaluated.

Using female NZB/WF1 mice, silastic implants containing the powdered form of endocrine disruptors were placed subcutaneously on the back of ovariectomized mice. The implants were left in situ for 3 to 4 months and blood samples were collected periodically, and anti-DNA antibody was measured in ELISA using dsDNA (Yurino H. 2004).

[*in vitro* assay]

The amounts of anti-dsDNA, anti-glomerular antigens (GA), total IgG and IgM in the culture supernatants were measured by ELISA (Kanda N. 1999, Wu WM. 2000, Yurino H. 2004, Gabriela T. 2019, John LS. 2008, Wang Y.1996). Proliferative responses PBMCs or B cells were measured by [3H]-thymidine uptake, and the cell viability was assessed by a trypan blue exclusion test (Kanda N. 1999). Fluorescence activated cell sorting (FACScan) was used for the quantitated of total B cells and CD5+B cells expression in spleen and in peritoneal exudates or B cell subset analysis (Wu WM. 2000, Peeva E. 2005). Plaque forming cell (PFC) assay using autologous bromelain-treated erythrocytes (Br-RBC) was conducted to examine the effect of EDs on autoantibody production by B1 cells (Yurino H. 2004).

Enzyme-linked immunospot (ELISPOT) analysis confirmed a significant increase in the number of high-affinity anti-DNA antibody-secreting B cells in the spleens of E2-treated mice (Bynoe MS. 2000).

References

1. Daniel, P., Allison, S., Yiming, Y., Ying-Yi, Z. and Laurence, M. Murine Models of Systemic Lupus erythematosus. Journal of Biomedicine and Biotechnology 2011: ArticleID 271694
2. Wu WM., Lin, B.-F., Su, Y.-C., Suen, J.-L. Chiang, B.-L. (2000). Tamoxifen decreases renal inflammation and alleviates disease severity in autoimmune NZB/W F1 mice. Scandinavian Journal of Immunology 52(4): 393-400.
3. Peeva, E., Venkatesh, J. and Diamond, B. (2005). Tamoxifen Blocks Estrogen-Induced B Cell Maturation but Not Survival. The Journal of Immunology 175: 1415-1423.
4. Bynote, KK., Hackenberg, J. M., Korach, K.S., Lubahn, D. B., Lane, P. H.and Gould, K. A. (2008). Estrogen receptor-alpha deficiency attenuates autoimmune disease in (NZB xNZW) F1 mice. Genes and Immunity. 9: 137-152.
5. Isenberg, DA., Manson, JJ., Ehrenstein, MR. and Rahman, A. (2007). Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology 46:1052-6.
6. Yurino, H., Ishikawa, S., Sato, T., Akadegawa, K., Ito, T., Ueha, S., Inadera, H. and Matsushima, K. (2004). Endocrine disruptors (environmental estrogens) enhance autoantibody production by B1 cells. Toxicological Sciences 81(1): 139-147.
7. Kanda N. and Tamaki, K. (1999). Estrogen enhances immunoglobulin production by human PBMCs. The Journal of Allergy and Clinical Immunology 103(2): 282-288.
8. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen alters thresholds for B cell apoptosis and activation. J Clin Invest. 2002;109(12):1625-33.
9. Peeva E, Grimaldi C, Spatz L, Diamond B. Bromocriptine restores tolerance in estrogen-treated mice. J Clin Invest. 2000;106(11):1373-9.
10. Gabriela, T., Yessia, H., Maria, R. B. and Mario, R. (2019), A Spontaneous Mouse Model of Lupus: Physiology and Therapy. IntechOpen Limited: 1-24.
11. John, L. S., Jackie, E., Phil, R., Kenneth, S. K. and Gary, S. G. (2008), Impact of estrogen receptor deficiency on disease

expression in the NZM2410 lupus prone mouse. Clin Immunol. 128(2): 259-268.
12. Wang, Y., Hu, Q., Madri, J. A., Rollins, S.A., Chodera, A, and Matis, L. A. (1996), Amelioration of lupus-like autoimmune disease in NZB/W F1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A. 93(16):8563-8568.
13. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates Bcl-2 and blocks tolerance induction of naı¨ve B cells. PNAS 2000; 97(6):2703-8.

List of Adverse Outcomes in this AOP

Event: 1714: Exacerbation of systemic lupus erythematosus (SLE)

Short Name: Exacerbation of SLE

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:314 - Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	AdverseOutcome

Stressors

Name
17beta-Estradiol
Bisphenol A

Biological Context

Level of Biological Organization
Individual

Evidence for Perturbation by Stressor

17beta-Estradiol

The NZB/W F1 mouse is the oldest classical model of lupus generated by the F1 hybrid between the NZB and NZW strains. In both NZB/W F1 and MRL/lpr mice, estrogen treatment exacerbates the lupus disease (Grimaldi CM. 2002, Peeva E. 2000). In postmenopausal women there was an increase in number of mild flares in women receiving estrogen supplementation suggesting that the addition of estrogen to a low estrogen state enhances flare rate (Buyon JP. 1998).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Mixed	

Exacerbation of SLE is common in humans and rodents, and is considered likely to occur in other animal species, as well. SLE is an autoimmune disease that occurs primarily in women (9:1 compared to men) (Rider V. 2001). SLE is an autoimmune disease that affects predominantly women during reproductive years, and its evolution is altered by hormonal events such as menses, menopause, and especially pregnancy (Luis JJ. 2014). The incidence of SLE is markedly increased in females of child-bearing age (Grainne M. 2013). Th1/Th2 shift is one of the most important immunologic changes during gestation. It is due to the progressive increase of estrogens, which reach peak level in the third trimester of pregnancy. At these high levels, estrogens suppress the Th1-mediated responses and stimulate Th2-mediated immunologic responses. For this reason, Th1-mediated diseases, such as rheumatoid arthritis, tend to improve, while Th2-mediated diseases, such as SLE tend to worsen during pregnancy (Doria A. 2006).

Female MRL/lpr mice that developed lymphadenopathy and a lupus-like disease also exhibited a 50% higher mortality rate than males at 5 months of age. In (NZB×NZW) F1 mice too, females develop signs of SLE several months before males, with severe autoimmune hemolytic anemia, glomerulonephritis, and autoantibodies to single-stranded DNA, doublestranded DNA, and histones (Carlsten H. 1992).

The effects of estrogen receptor signaling on T cells also appear to be dose dependent (Melissa, and Gary 2011). Low serum levels (60-100 pg/mL or 0.26-0.43 nM) of estradiol have been shown to increase Th1 T-cell development in vitro through an ERα mediated mechanism (Maret A. 2003). In contrast of SLE exacerbated by Th2, treatment with low doses of estrogen (25 pg/ml or 0.1 nM) ameliorated autoimmune diseases (multiple sclerosis; MS, rheumatoid arthritis; RA, and experimental autoimmune encephalomyelitis; EAE, etc.) caused by Th1, while high doses (>1000 pg/ml or 4.3 nM), which mimic pregnancy levels, prevented EAE onset polarized T-cells to a Th2 phenotype in the EAE model (Bebo BF. 2001).

Key Event Description

SLE is an autoimmune disease characterized by overproduction of a variety of anti-cell nuclear and other pathogenic autoantibodies. It is characterized by B-cell hyperactivity, polyclonal hypergammaglobulinemia, and glomerulonephritis as immune complex deposition. Once SLE is suspected, the initial evaluation should include an antinuclear antibody (ANA) test. This is a highly sensitive test, with positive results in about 94% of patients with SLE. However, it also has low specificity, and may be positive in healthy patients. If ANA results show a 1:40 titer or higher, more specific tests should be performed, including measurement of anti–double-stranded DNA (anti-dsDNA), anti-Smith, anti-RNP, anticardiolipin, beta-2 glycoprotein antibodies and lupus anticoagulant; elevated levels of one or more of these biomarkers increase the likelihood of SLE (Nguyet-Cam VL. 2016). In the Systemic Lupus International Collaborating Clinics 2012 classification for SLE, biopsy-proven lupus nephritis plus positive ANA or anti-dsDNA is sufficient to fulfil SLE classification criteria (Bernard T. 2017). SLE is the prototypic multisystem autoimmune disorder with a broad spectrum of clinical presentations encompassing almost all organs and tissues including skin, kidney, heart, lungs, and joints. The pathogenesis of SLE includes both genetic and environmental components with female sex strongly influencing pathogenesis. These factors lead to an irreversible break in immunological tolerance manifested by immune responses against endogenous nuclear antigens (Daniel P. 2011).

It has been determined in a murine model of SLE that ERα is required for disease progression and that ERα deficiency impedes the course of the disease (Bynote KK. 2008). There is increased ERα mRNA expression in PBMCs of SLE patients (Inui A. 2007). It is considered that MIE affect later events and result in SLE.

How it is Measured or Detected

[*in vivo* assay]

Murine lupus models such as New Zealand Black (NZB)×New Zealand White (NZW) F1 (NZB/W F1), NZB.H-2bm12, NZB×SWR F1 (SNF1), MRL.lpr/lpr, and BXSB mice have led to a better understanding of the pathogenic mechanisms of lupus. All of these species of mice develop anti-dsDNA antibody, which is a characteristic of lupus, and die of uremia in early life. Among these murine lupus models, the natural course of NZB/W F1 mice is closer to human lupus than MRL.lpr/lpr and BXSB mice (Zhang DH. 1997, Pai SY. 2004, Daniel P. 2011).

For the disease onset, mice can monitor by proteinuria levels, body weights, blood urea nitrogen and appearance over time. (Gabriela T. 2019, John LS. 2008, Wang Y.1996). The major cause of death in the NZB/W F1 female is chronic glomerulonephritis with heavy mesangial deposits, tubular cast formation, proliferation of glomerular cells, prominent crescent formation, and a significant periglomerular and interstitial monocytic infiltrate. Extraglomerular renal deposits of IgG2a and C3 are present in the peritubular tissue and arterioles, and increase in frequency with age. Histological alterations in the kidney were assessed by Hematoxylin Eosin (H&E) and Periodic acid-Schiff (PAS) staining, expression of IgG and C3 was detected by immunohistochemistry (Gabriela T. 2019, Brian S. 1978).

To examine the relationship between oral contraceptive (OC) use and the development of SLE, analyzed data (1976 - 1990) from the Nurses' Health Study cohort. The questionnaire used to assemble biennially the group sought information on a variety of health conditions and exposures, such as use of OCs, use of post-menopausal hormones (PMH), current and past cigarette smoking habits and other health practices. Incidence of SLE was defined by; 1) strict American College of Rheumatology (ACR) classification criteria (> or = 4 ACR criteria), 2) > or = 4 ACR criteria and any physician's diagnosis, 3) > or = 4 ACR criteria and diagnosis by an ACR-certified rheumatologist, 4) > or = 3 ACR criteria, or 5) diagnosis by a physician even if the patient did not meet the ACR criteria. (Bertsias G. 2012, Sanchez-Guerrero J.1997).

Typical clinical symptoms include combinations of renal disease, swollen joints, skin rash, hematologic disorders, respiratory, and neurologic dysfunction.

Regulatory Significance of the AO

There are concerns about the increase in autoimmune diseases caused by estrogen-like substances, and its accurate in vitro toxicity assessment system is required in international regulations. The OECD has published a revised version of the guidance document on standardized test guidelines for evaluating ED (OECD. 2019).

References

1. Nguyet-Cam Vu Lam, Maria V. Ghetu and Marzena L. BIENIEK. Systemic Lupus Erythematosus: Primary Care Approach to Diagnosis and Management. American Family Physician, 2016; 94 (4): 284-294.
2. Bernard Thong and Nancy J. Olsen. Systemic lupus erythematosus diagnosis and management. Rheumatology 2017; 56: i3-i13.
3. Daniel, P., Allison, S., Yiming, Y., Ying-Yi, Z. and Laurence, M. Murine Models of Systemic Lupus erythematosus. Journal of Biomedicine and Biotechnology 2011: ArticleID 271694
4. Bynote, KK, Hackenberg, JM., Korach, K.S., Lubahn, D. B., Lane, P. H. and Gould, K. A. (2008). Estrogen receptor-alpha deficiency attenuates autoimmune disease in (NZB xNZW) F1 mice. Genes and Immunity. 9: 137-152.
5. Inui A, Ogasawara H, Ogawa H, et al. Estrogen receptor expression by peripheral blood mononuclear cells of patients with systemic lupus erythematosus. Clin Rheumatol. 2007;26(10):1675-8.
6. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997. 22;272(34):21597-603.
7. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc Natl Acad Sci U S A. 2004 Feb 17;101(7):1993-8.
8. Gabriela, T., Yessia, H., Maria, R. B. and Mario, R. (2019), A Spontaneous Mouse Model of Lupus: Physiology and Therapy. IntechOpen Limited: 1-24.
9. John, L. S., Jackie, E., Phil, R., Kenneth, S. K. and Gary, S. G. (2008), Impact of estrogen receptor deficiency on disease expression in the NZM2410 lupus prone mouse. Clin Immunol. 128(2): 259-268.
10. Wang, Y., Hu, Q., Madri, J. A., Rollins, S.A., Chodera, A, and Matis, L. A. (1996), Amelioration of lupus-like autoimmune disease in NZB/W F1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. Proc Natl Acad Sci U S A. 93(16):8563-8568.
11. Brian S. Andrews, Robert A. Eisenberg, Argyrios N. Theofilopoulos, S Izui, Curtis B. Wilson, Patricia J. McConahey, Edwin D. Murphy, John B. Roths and Frank J. Dixon. Spontaneous Murine Lupus-Like Syndromes. Clinical and Immunopathological Manifestations in Several Strains. J. EXP. Med. 1978; 148(5):1198-215
12. Bertsias G, Ricard Cervera and Dimitrios T. Boumpas. Systemic Lupus Erythematosus: Pathogenesis and Clinical Features. 20_Eular_Fpp.indd. 2012; 476-505.
13. Sanchez-Guerrero J. Karlson EW. Liang MH. Hunter DJ,. Speizer F. E, and Colditz. G. A. Past Use of Oral Contraceptives and the Risk of Developing Systemic Lupus Erythematosus. Arthritis Rheum. 1997; 40 (5): 804-808.
14. Rider, V. and Abdou, N. I. (2001). Gender differences in autoimmunity: molecular basis for estrogen effects in systemic lupus erythematosus. International Immunopharmacology 1(6): 1009-1024.
15. Luis, J. J., Gabriela, M., Pilar, C.-D., Carmen, N., Olga V.-L. and Miguel., A. S. (2014). Risk factors of systemic lupus erythematosus flares during pregnancy. Immunologic Research 60: 184-192
16. Grainne, M. and David, I. (2013). Effect of gender on clinical presentation in systemic lupus erythematosus. Rheumatology 52: 2108-2115
17. Doria, A., Iaccarino, L., Sarzi-Puttini, P., Ghirardello, A., Zampieri, S., Arienti, S., Cutolo, M. and Todesco, S. (2006). Estrogens in pregnancy and systemic lupus erythematosus. Annals of the New York Academy of Sciences 1069: 247-256.
18. Carlsten H, Nilsson N, Tarkowski A, et al. Estrogen accelerates immune complex glomerulonephritis but ameliorates T cell-mediated vasculitis and sialadenitis in autoimmune MRL lpr/lpr mice. Cell Immunol. 1992;144(1):190-202.
19. Melissa, C and Gary, G (2011). Estrogen Receptors in Immunity and Autoimmunity. Clinical Reviews in Allergy & Immunology 40: 66-73.
20. Maret, A., Coudert, J. D., Garidou, L., Foucras, G., Gourdy, P., Krust, A., Dupont, S., Chambon, P., Druet, P., Bayard, F. and Guéry, J. C. (2003). Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. The European Journal of Immunology 33: 512-521.
21. Bebo, B. F. Jr., Fyfe-Johnson, A., Adlard, K., Beam, A. G., Vandenbark, A. A.and Offner, H. Low-Dose Estrogen Therapy Ameliorates Experimental Autoimmune Encephalomyelitis in Two Different Inbred Mouse Strains. (2001). The Journal of Immunology. 166: 2080-2089.
22. Grimaldi CM, Cleary J, Dagtas AS, Moussai D, Diamond B. Estrogen alters thresholds for B cell apoptosis and activation. J Clin Invest. 2002;109(12):1625-33.
23. Peeva E, Grimaldi C, Spatz L, Diamond B. Bromocriptine restores tolerance in estrogen-treated mice. J Clin Invest. 2000;106(11):1373-9.
24. Buyon JP. Hormone replacement therapy in postmenopausal women with systemic lupus erythematosus. J Am Med Womens Assoc (1998) 53(1):13-17.
25. OECD Series on Testing and Assessment [Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption. 2019].

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2020: Binding to estrogen receptor (ER)-α leads to Induction of GATA3 expression

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

XXXX

Key Event Relationship Description

The hormone binding domain (HBD) of the ERα is required not only for binding ligand but also to form stable homodimers of the protein and mediate transcriptional activation by the receptor. There are two ligand-dependent signaling pathway. One is “classical” and the other is “tethered” pathway. A direct genomic interaction occurs between the ER ligand complex and specific sequences of DNA known as estrogen response elements (ERE) (Parker MG. 1993, Goldstein RA. 1993, Sasson S. 1991, Brandt ME. 1997). Transcriptional activation by ERα is mediated by two distinct activation functions: the constitutively active AF-1 domain, located in the N-terminal domain of the receptor protein, and the ligand-dependent AF-2 domain, located in the C-terminal domain of the receptor protein (Delaunay F. 2000). This is called “classical” signaling pathway. In addition to above classical mechanism, ligand-activated ERα interact with other transcription factor complexes and bind to non-EREs by attaching to other transcription factors and not with ERE directly. (Carolyn MK. 2001). This is also called “tethered” signaling pathway. The transcription factors GATA3 and STAT6 are essential for the establishment and/or maintenance of these interactions (Spilianakis and Flavell, 2004). In the tethered pathway, STAT6-ER fusion protein induce GATA-3 mRNA expression. Furthermore, in mammary gland but not in immune cells, GATA3 and ERα regulate each other and, along with FOXA1, can nucleate a remodeling complex at heterochromatic enhancer regions of ERα target genes, leading to the opening and epigenetic marking of sites for active transcription (Eeckhoute J. 2007, Kong SL. 2011). Alone, FOXA1 or ERα are not sufficient to fully open the chromatin, supporting a bona fide pioneer activity for GATA3 (Eeckhoute J. 2007, Kong SL. 2011).

Evidence Supporting this KER

Biological Plausibility

STAT6-ER fusion protein (STAT6:ER) induce expression of GATA-3 mRNAs in presence of 4-Hydroxytamoxifen (4-HT), estrogen analogue (Kurata H. 1999, Zhu J. 2001). Furthermore, A constitutively active form of Stat6 (STAT6VT) introduced GATA3 expression and resulted in both Th2 differentiation and enhanced cell expansion without IL-4 (Zhu J. 2001, Horiuchi S. 2011). CD4 T cells from Stat6-knockout mice are not able to drive Th2 differentiation and cell expansion under ThN conditions with added IL-4 (Zhu J. 2001). Therefore, it is considered that activated STAT6 after ligand-biniding to ERα induce GATA3 expression in immune cells.

Empirical Evidence

Expression of GATA3 was induced in T cells treated with E_2 at a concentration of 10^{-9} M (272.4 pg/mL) for 12-16 hours (Lambert KC. 2005). In contrast, expression of T-bet was decreased, which means E_2 skew the immune system from a Th1 to a Th2 profile. Stat6:ER Th1 cells expressed significant amounts of both GATA3 mRNAs in a 4-HT-dependent manner (Kurata H. 1999, Zhu J. 2001). Constitutively activated Stat6 (Stat6VT) is primed under null Th cell (ThN) conditions in the absence of human (h)IL-4. The expression level of Gata3 in this primed cells are checked by RT-PCR (Zhu J. 2001).

M12.4.1 cells, transfected with the luciferase reporter gene by inserting three copies of human STAT6 binding site oligonucleotide, are used nuclear extracts and electrophoretic mobility shift assay (EMSA) with 1 μM 4-HT. STAT6:ER DNA-binding activity is strongly and rapidly (within 1 hr) induced after addition of 4-HT to these cells. BA/F3 cells prepared as the same manner are stimulated with 1 μM 4-HT for 24 h at 37°C. The cells were harvested and assayed for luciferase activities using a Luciferase Assay Kit (Kamogawa Y. 1998).

Uncertainties and Inconsistencies

The “tethered” pathway is confirmed indirectly using artificial STAT6-ER fusion protein but not endogenous STAT6. It remains unknown whether the “classical” pathway is utilized after binding to ERα in immune cells.

Quantitative Understanding of the Linkage

Response-response relationship

MIE:

XXXX

KE XX:

XXXX

Time-scale

XXXX

Known modulating factors

The Th1/Th2 shift is one of the most important immunologic changes during the menstrual cycle and gestation. Immune activity shifts across the menstrual cycle, with higher follicular-phase Th1 cell activity and higher luteal-phase Th2 cell activity (Tierney KL. 2015). This is due to the progressive increase of estrogens, which reach peak level in the third trimester of pregnancy. At these high levels, estrogens suppress the Th1-mediated responses and stimulate Th2-mediated immunologic responses (Doria A. 2006). The effects of ERα signaling on T cells appear to be estrogen-dose dependent, i.e., low doses of estrogen stimulate a Th1 response, but higher doses promote a Th2 response (Priyanka HP. 2013).

Known Feedforward/Feedback loops influencing this KER

XXXX

References

1. Parker MG, Arbuckle N, Dauvois S, Danielian P, White R. Structure and function of the estrogen receptor. Ann N Y Acad Sci. 1993. 684:119-26.
2. Goldstein RA, Katzenellenbogen JA, Wolynes PG, et al. Three-dimensional model for the hormone binding domains of steroid receptors. Proc Natl Acad Sci. 1993;90 (21):9949-53.
3. Sasson S. Equilibrium binding analysis of estrogen agonists and antagonists: relation to the activation of the estrogen receptor. Pathol Biol (Paris). 1991;39(1):59-69.
4. Brandt ME, Vickery LE. Cooperativity and dimerization of recombinant human estrogen receptor hormone-binding domain. J Biol Chem. 1997;272(8):4843-9.
5. Delaunay, F., Pettersson, K., Tujague, M., and Gustafsson, J. A. (2000). Functional Differences between the Amino-Terminal Domains of Estrogen Receptors α and β. Molecular Pharmacology 58: 584-590.
6. Carolyn MK. Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res. 2001 Jul 15; 29(14): 2905-2919.
7. Spilianakis CG & Flavell RA, Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. Nature Immunology. 2004; 5: 1017-1027.
8. Eeckhoute J, Positive Cross-Regulatory Loop Ties GATA-3 to Estrogen Receptor α Expression in Breast Cancer. Cancer Res. 2007; 67(13):6477-83.
9. Kong SL, Cellular reprogramming by the conjoint action of ERα, FOXA1, and GATA3 to a ligand-inducible growth state. Mol Syst Biol (2011)7:526
10. Kurata, H., Lee, H. J., O'Garra, A. and Arai, N. (1999). Ectopic expression of activated STAT6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 11: 677-688.
11. Zhu, J., Guo, L., Watson, C. J., Hu-Li, J. and Paul, W. E. (2001). STAT6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. The Journal of Immunology 166(12): 7276-7281.
12. Horiuchi S, Genome-wide analysis reveals unique regulation of transcription of Th2-specific genes by GATA3. (2011) J Immunol. 1;186(11):6378-89.
13. Lambert KC, Curran EM, et al. Estrogen receptor alpha (ERalpha) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17beta-estradiol acts through ERalpha to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol. 2005; 175(9): 5716-23.
14. Kamogawa, Y., Lee, H.J., Johnston, J.A., McMahon, M., O'Garra, A., and Arai, N. (1998). Cutting Edge: A conditionally active form of STAT6 can mimic certain effects of IL-4. J. Immunol. 161, 1074-1077.
15. Tierney, K. L., Julia, R. H. and Gregory, E. D. (2015). Sexual activity modulates shifts in Th1/Th2 cytokine profile across the menstrual cycle: An observational study. Fertility and Sterility 104 (6): 1513-1521.
16. Doria, A., Iaccarino, L., Sarzi-Puttini, P., Ghirardello, A., Zampieri, S., Arienti, S., Cutolo, M. and Todesco, S. (2006). Estrogens in pregnancy and systemic lupus erythematosus. Annals of the New York Academy of Sciences 1069: 247-256.
17. Priyanka HP, Krishnan HC, Singh RV, Hima L, Thyagarajan S. Estrogen modulates in vitro T cell responses in a concentration- and receptor-dependent manner: effects on intracellular molecular targets and antioxidant enzymes. Mol Immunol. 2013;56(4):328-39.

Relationship: 2021: Induction of GATA3 expression leads to Increase of Th2 cells producing IL-4

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

XXXX

Key Event Relationship Description

Intrachromosomal interactions in the Th2 cytokine locus may form the basis for the coordinated transcriptional regulation of cytokine-encoding genes by the Th2 locus control region (Spilianakis and Flavell, 2004). During Th2 cell differentiation, binding patterns of PcG and TrxG proteins are dynamically changed at the *Gata3* gene locus, and these epigenetic changes result in GATA3 protein upregulation, which consequently induces chromatin remodeling at the Th2 cytokine gene loci, including *Il4*, *Il5*, and *Il13* (Ansel KM. 2006, Horiuchi S. 2011).

Evidence Supporting this KER

Biological Plausibility

Th2 differentiation is completely abolished both in vitro and in vivo when GATA3 is conditionally deleted in peripheral CD4 T cells. Th2 cells from both knockout animals showed reduction in IL-4 production. (Zhu J. 2004, Pai SY. 2004).

The GATA3 expression induced by TNF-α was enhanced in the presence of BPA. However, the T-bet expression did not change when tested at various culture conditions (Guo H. 2010, Uemura Y. 2008). IL-4 may serve multiple roles in the development of lupus: it may enhance autoantibody production via its direct B-cell effects (Ram RS. 2003).

Empirical Evidence

The proliferation of Stat6:ER Th2 cells was enhanced in a dose-dependent manner on days 10 and 31 after polarization by [^{3}H]thymidine incorporation (the effective concentration of 4-HT was between 0.08 and 2 μM, and the toxic concentration was greater than 5 μM) (Kurata H. 1999, Zhu J. 2001). Purified naive T cells were activated and infected with RV-Stat6:ER. The cells were cultured and expanded under Th culture conditions in the presence or absence of 0.3 μM 4-HT (Research Biochemicals Institute) for 2 weeks starting from days 1, 7, 14, or 21 and the cells were analyzed for cytokine (IL-4) expression by flow cytometer analysis of intracellular cytokine production or cytokine ELISA (Kurata H. 1999, Zhu J. 2001). CD4 T cells from Stat6-knockout mice are not able to drive Th2 differentiation and cell expansion under null Th cell (ThN) conditions with added with IL-4 (Zhu J. 2001).

Th2 differentiation is completely abolished both in vitro and in vivo when GATA3 is conditionally deleted in peripheral CD4 T cells from GATA-3-deficient (FF and FF cre) mice (Sung-Yun. 2004, Zhu J. 2004). Antigen-specific immune response is evaluated with lymphocyte from FF and FF cre mice injected with KLH, and cytokine production was measured by sandwich ELISA (Sung-Yun. 2004).

Quantitative Understanding of the Linkage

The effects of estrogen receptor signaling on T cells also appear to be dose dependent (Cunningham M. 2011). When estrogen levels are low, T cell expansion shift toward a Th1 phenotype that produces IL-12, TNF-α, and IFN-γ.

Response-response relationship

MIE:

XXXX

KE XX:

XXXX

Time-scale

XXXX

Known modulating factors

The Th1/Th2 shift is one of the most important immunologic changes during gestation. This is due to the progressive increase of estrogens, which reach peak level in the third trimester of pregnancy. At these high levels, estrogens suppress the Th1-mediated responses and stimulate Th2-mediated immunologic responses (Doria et al. 2006).

Known Feedforward/Feedback loops influencing this KER

XXXX

References

1. Spilianakis CG & Flavell RA, Long-range intrachromosomal interactions in the T helper type 2 cytokine locus. Nature Immunology. 2004; 5: 1017-1027.
2. Ansel KM, Djuretic I, Tanasa B, RaoA. 2006. Regulation ofTh2 differentiation and *Il4* locus accessibility. *Annu. Rev. Immunol.* 24:607-56.
3. Horiuchi S, Onodera A, Hosokawa H, Watanabe Y, Tanaka T, et al. 2011. Genome-wide analysis reveals unique regulation of transcription of Th2-specific genes by GATA3. *J. Immunol.* 186:6378-89.
4. Zhu J, Min B, Paul WE, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5(11):1157-65.
5. Pai SY, Truitt ML, Ho IC. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proc Natl Acad Sci U S A. 2004 Feb 17;101(7):1993-8.
6. Guo H, Liu T, Ling F, et al. Bisphenol A in combination with TNF-alpha selectively induces Th2 cell-promoting dendritic cells in vitro with an estrogen-like activity. Cell Mol Immunol. 2010;7(3):227-34.
7. Uemura Y, Liu TY, Narita Y, Suzuki M, Matsushita S. 17 Beta-estradiol (E2) plus tumor necrosis factor-alpha induces a distorted maturation of human monocyte derived dendritic cells and promotes their capacity to initiate T-helper 2 responses. Hum Immunol. 2008;69(3):149-57.
8. Ram Raj Singh (2003). IL-4 and many roads to lupuslike autoimmunity. Clinical Immunology 108: 73-79.
9. Kurata, H., Lee, H. J., O'Garra, A. and Arai, N. (1999). Ectopic expression of activated STAT6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. Immunity 11: 677-688.
10. Zhu, J., Guo, L., Watson, C. J., Hu-Li, J. and Paul, W. E. (2001). STAT6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. The Journal of Immunology 166(12): 7276-7281.
11. Sung-Yun, Morgan L. T. I-Cheng H. (2004). GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proceedings of the National Academy of Sciences. 101 (7): 1993-1998.
12. Zhu J, Min B, Paul WE, et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat Immunol. 2004;5(11):1157-65.
13. Cunningham, M., Gilkeson, G., 2011. Estrogen receptors in immunity and autoimmunity. Clinical Reviews in Allergy and Immunology 40, 66-73.
14. Doria, A., Iaccarino, L., Sarzi-Puttini, P., Ghirardello, A., Zampieri, S., Arienti, S., Cutolo, M. and Todesco, S. (2006). Estrogens in pregnancy and systemic lupus erythematosus. Annals of the New York Academy of Sciences 1069: 247-256.

Relationship: 2022: Increase of Th2 cells producing IL-4 leads to Increase of autoantibody production

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	adjacent	Moderate	Moderate

Key Event Relationship Description

During process of B cell maturation, the autoreactive B cell which has high-affinity for DNA are normally silenced by anergy, and activated by stimulation with antigen independent CD40 ligand (CD154) or IL-4 from Th2 cells. The receptor for IL-4 is IL-4Rα, which expresses in B cells. In the development of T-cell dependent antibody producing cells, the interaction between IL-4 and its receptor stimulates B-cells to mature (proliferate, switch immunoglobulin classes). As a result, production of anti-DNA antibody from activated autoreactive B cells in increased.

Evidence Supporting this KER

Biological Plausibility

Lack of ERα, in either male or female mice, did not increase B cell precursors (Smithson G. 1998). Restoration of estradiol in ovariectomized NZB/W F1 mice reestablished high numbers of autoantibody-producing (DNA-specific) B cells, and thereby suggests a pathogenic role of estrogen in lupus (Daniel P. 2011).

Anergic B cells, dsDNA-specific models, can be stimulated by IL-4 specific antibody in vitro, suggesting that they are capable of responding to T-cell-derived signals (Acevedo-Suarez CA. 2005, Noorchashm H. 1999, Mandik-Nayak L. 2000, Eris JM. 1994).

Transfer of either IL-4-stimulated splenocytes from 5-mo-old NZB/W F1 mice into NZB/W F1 mice of the same age enhanced the production of IgG anti-dsDNA Ab. Consistently, administration of mAb against IL-4 before the onset of lupus was effective in preventing the onset of lupus nephritis (Nakajima A. 1997).

Empirical Evidence

The administration of the estrogen antagonist tamoxifen diminishes anti-DNA antibody levels by ELISA as well as decreases percentages of total B cells and CD5+ B cells by FCM (Wu WM. 2000). Tamoxifen blocks estrogen-induced B cell maturation but not survival (Peeva E. 2005). ERα deficiency in (NZB×NZW) F1 female mice downregulated levels of anti-dsDNA IgG antibodies,

and the absence of ERα In (NZB×NZW) F1 males resulted in decreased anti-dsDNA antibodies (Bynote KK. 2008).

Uncertainties and Inconsistencies

Estrogen upregulates CD40L (CD154) on T cells from SLE patients (Desai-Mehta A. 1996, Li X. 2006). Anti-CD40L antibodies downregulate CD86 expression on normal and SLE B lymphocytes, blockade of CD86 only diminishes anti-DNA antibody production by SLE B cells (Nagafuchi H. 2003). Moreover, mice overexpressing CD40L develop a lupus-like disease with high levels of antibodies to nuclear antigens, DNA, and histones, as well as glomerulonephritis (Higuchi T. 2002). Activation of autoreactive B cell may be involved in stimulation not only IL-4, but also CD40 ligand (CD154) of Th2 cell as well as the other immune cells.

B1 cells from aged mice exhibited increased expression of ERα mRNA compared to young mice (Yurino H. 2004). Since the ER of B cell is also expressed, there may be a direct route that does not go through Th2.

Quantitative Understanding of the Linkage

Response-response relationship

MIE:

XXXX

KE XX:

XXXX

Time-scale

XXXX

Known modulating factors

XXXX

Known Feedforward/Feedback loops influencing this KER

XXXX

References

1. Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. J Immunol. 1998;161(1):27-34.
2. Daniel, P., Allison, S., Yiming, Y., Ying-Yi, Z. and Laurence, M. Murine Models of Systemic Lupus erythematosus. Journal of Biomedicine and Biotechnology 2011: ArticleID 271694
3. Acevedo-Suarez CA, Hulbert C, Woodward EJ, Thomas JW. Uncoupling of anergy from developmental arrest in anti-insulin B cells supports the development of autoimmune diabetes. J. Immunol. 2005; 174:827-833.
4. Noorchashm H, et al. Characterization of anergic anti-DNA B cells: B cell anergy is a T cellindependent and potentially reversible process. Int. Immunol. 1999; 11:765-776.
5. Mandik-Nayak L, et al. Functional consequences of the developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. J. Immunol. 2000; 164:1161-1168.
6. Eris JM, et al. Anergic self-reactive B cells present self-antigen and respond normally to CD40-dependent T-cell signals but are defective in antigen-receptor-mediated functions. Proc. Natl Acad. Sci. USA. 1994; 91:4392-4396.
7. Nakajima A, Hirose S, Yagita H and Okumura K, Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. J Immunol 1997; 158 (3) 1466-1472.
8. Wu WM., Lin, B.-F., Su, Y.-C., Suen, J.-L. and Chiang, B.-L. (2000). Tamoxifen decreases renal inflammation and alleviates disease severity in autoimmune NZB/W F1 mice. Scandinavian Journal of Immunology 52(4): 393-400.
9. Peeva, E., Venkatesh, J. and Diamond, B. (2005). Tamoxifen Blocks Estrogen-Induced B Cell Maturation but Not Survival. The Journal of Immunology 175: 1415-1423.
10. Bynote, KK. Hackenberg, JM., Korach, KS, Lubahn, DB., Lane, PH.and Gould, KA. (2008). Estrogen receptor-alpha deficiency attenuates autoimmune disease in (NZB xNZW) F1 mice. Genes and Immunity. 9: 137-152.
11. Desai-Mehta A, Lu L, Ramsey-Goldman R, Datta SK. Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. J Clin Invest. 1996. 1;97(9):2063-73.
12. Li X, Rider V, Kimler BF, Abdou NI. Estrogen does not regulate CD154 mRNA stability in systemic lupus erythematosus T cells. Lupus. 2006;15(12):852-7.
13. Nagafuchi H, Shimoyama Y, Suzuki N, et al. Preferential expression of B7.2 (CD86), but not B7.1 (CD80), on B cells induced by CD40/CD40L interaction is essential for anti-DNA autoantibody production in patients with systemic lupus erythematosus. Clin Exp Rheumatol. 2003;21(1):71-7.
14. Higuchi T, Aiba Y, Tsubata T. Cutting Edge: ectopic expression of CD40 ligand on B cells induces lupus-like autoimmune disease. J Immunol. 2002. 1;168(1):9-12.
15. Yurino, H., Ishikawa, S., Sato, T., Akadegawa, K., Ito, T., Ueha, S., Inadera, H. and Matsushima, K. (2004). Endocrine disruptors (environmental estrogens) enhance autoantibody production by B1 cells. Toxicological Sciences 81(1): 139-147.

Relationship: 2023: Increase of autoantibody production leads to Exacerbation of SLE

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Binding to estrogen receptor (ER)-α in immune cells leading to exacerbation of systemic lupus erythematosus (SLE)	adjacent	Moderate	Moderate

Evidence Supporting Applicability of this Relationship

XXXX

Key Event Relationship Description

The presence of many autoantibodies is a hallmark of SLE. In particular, autoantibodies directed to double-stranded DNA (dsDNA) are characteristic (Isenberg DA. 2007). SLE patients appear to produce significant amounts of the anti-dsDNA autoantibodies that cause the disease. Anti-dsDNA antibody exists even in healthy people, but in SLE patients, an increase in anti-dsDNA antibody has been observed and is also used for definitive diagnosis of SLE. Activation of autoantibody-producing B cells only serves to exacerbate that condition.

Evidence Supporting this KER

Biological Plausibility

SLE has been seen to flare up during pregnancy (Petri M. 1991). The aberrant T cell dysfunction in SLE is also associated with high levels of autoantibodies (Crispin JC. 2010).

Premenopausal women receiving low estrogen containing birth control pills did not have an increased flare rate compared to women receiving placebo suggesting that adding estrogen to an already high estrogen state had no effect on disease (Buyon JP. 1996).

Empirical Evidence

In a study to investigate a novel subpopulation of B-1 cells and its roles in murine lupus, anti-double-stranded DNA (anti-dsDNA) autoantibodies were preferentially secreted by a subpopulation of CD5+ B-1 cells that expressed programmed death ligand 2 (L2pB1 cells) (Xuemei Z. 2009). A substantial proportion of hybridoma clones generated from L2pB1 cells reacted to dsDNA. L2pB1 cells are potent antigen-presenting cells and a dramatic increase of circulating L2pB1 cells in lupus-prone BXSB mice correlates with elevated serum titers of anti-dsDNA antibodies (Xuemei Z. 2009).

Uncertainties and Inconsistencies

Stat6-deficient New Zealand Mixed (NZM) 2328 mice display a significant reduction in incidence of kidney disease, with a dramatic increase in survival, despite the presence of high levels of anti-dsDNA Abs same like the wild-type NZM 2328 animals (Chaim O. 2003). In NZM 2410 mice, STAT6 deficiency or anti-IL-4 Ab treatment decreases type 2 cytokine responses and ameliorates kidney disease, particularly glomerulosclerosis, despite the presence of high levels of IgG anti-dsDNA Abs same like the wild-type littermates or PBS-treated controls (Ram RS. 2003). Anti-dsDNA antibodies are not what we think they are, as they may be antibodies operational in quite different biological contexts, although they bind dsDNA by chance. This may not mean that these antibodies are not pathogenic but they do not inform how they are so (Ole PR. 2019). In other words, might be that the high levels of anti-dsDNA Abs does not always exacerbate SLE.

Quantitative Understanding of the Linkage

Response-response relationship

The effects of estrogen receptor signaling on T cells also appear to be dose dependent (Cunningham M. 2011). When estrogen levels are low, T cell expansion shift toward a Th1 phenotype that produces IL-12, TNF-α, and IFN-γ. This response results in cellular immunity inducing inflammation and exacerbating cellular type autoimmune diseases (multiple sclerosis; MS, rheumatoid arthritis; RA, and experimental autoimmune encephalomyelitis; EAE, etc.) caused by Th1 rather than SLE. Treatment with low serum levels (60-100 pg/mL or 0.26-0.43 nM) of estradiol increased Th1 T-cell development in vitro by acting through an ERα mediated mechanism (Maret A. 2003). Treatment with low doses of estrogen (25 pg/ml or 0.1 nM) ameliorated autoimmune diseases caused by Th1, while high dose levels (>1000 pg/ml or 4.3 nM), which mimic pregnancy levels, prevented EAE onset and polarized T-cells to a Th2 phenotype in the EAE. (Bebo BF. 2001, Korn-Lubetzki I. 1984).

Time-scale

XXXX

Known modulating factors

The Th1/Th2 shift is one of the most important immunologic changes during the menstrual cycle and gestation. Immune activity shifts across the menstrual cycle, with higher follicular-phase Th1 cell activity and higher luteal-phase Th2 cell activity (Tierney KL. 2015). This is due to the progressive increase of estrogens, which reach peak level in the third trimester of pregnancy. At these high levels, estrogens suppress the Th1-mediated responses and stimulate Th2-mediated immunologic responses (Doria A. 2006). Incidence of flare in patients with SLE is increased during pregnancy and within the 3-months postpartum (Amanda E. 2018).

Known Feedforward/Feedback loops influencing this KER

XXXX

References

1. Isenberg, DA., Manson, JJ., Ehrenstein, MR. and Rahman, A. (2007). Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology 46:1052-6.
2. Petri, M. Howard, D. and Repke, J. (1991). Frequency of lupus flare in pregnancy. The Hopkins Lupus Pregnancy Center experience. Arthritis & Rheumatology. 34(12): 1538-1545.
3. Crispin, JC. Liossis, SN. (2010). Pathogenesis of human systemic lupus erythematosus: recent advances. Trends in Molecular Medicine. 16: 47-57.
4. Buyon JP. Oral contraceptives in women with systemic lupus erythematosus. Ann Med Interne (Paris) (1996) 147(4):259-264.
5. Xuemei, Z., Stanley, L., et al. (2009). A Novel Subpopulation of B-1 Cells Is Enriched with Autoreactivity in Normal and Lupus-Prone Mice. Arthritis & Rheumatology 60 (12):3734-3743.
6. Chaim O. Jacob, Song Zang, Lily Li, Voicu Ciobanu, Frank Quismorio, Akiei Mizutani, Minoru Satoh and Michael Koss (2003). Pivotal Role of Stat4 and Stat6 in the Pathogenesis of the Lupus-Like Disease in the New Zealand Mixed 2328 Mice. J Immunol. 171 (3): 1564-1571.
7. Ram Raj Singh, Vijay Saxena, Song Zang, Lily Li, Fred D. Finkelman, David P. Witte and Chaim O. Jacob (2003). Differential Contribution of IL-4 and STAT6 vs STAT4 to the Development of Lupus Nephritis. J Immunol, 170 (9): 4818-4825
8. Ole Petter Rekvig (2019), The dsDNA, Anti-dsDNA Antibody, and Lupus Nephritis: What We Agree on, What Must Be Done, and What the Best Strategy Forward Could Be, Front. Immunol. 10: 1-17.
9. Cunningham, M., Gilkeson, G., 2011. Estrogen receptors in immunity and autoimmunity. Clinical Reviews in Allergy and Immunology 40, 66-73.
10. Maret, A., Coudert, J. D., Garidou, L., Foucras, G., Gourdy, P., Krust, A., Dupont, S., Chambon, P., Druet, P., Bayard, F. and Guéry, J. C. (2003). Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. The European Journal of Immunology 33: 512-521.
11. Bebo, B. F. Jr., Fyfe-Johnson, A., Adlard, K., Beam, A. G., Vandenbark, A. A.and Offner, H. Low-Dose Estrogen Therapy Ameliorates Experimental Autoimmune Encephalomyelitis in Two Different Inbred Mouse Strains. (2001). The Journal of Immunology. 166: 2080-2089.
12. Korn-Lubetzki, I., Kahana, E., Cooper, G. and Abramsky, O. (1984). Activity of multiple sclerosis during pregnancy and puerperium. Annals of Neurology 16(2): 229-231.
13. Maret, A., Coudert, J. D., Garidou, L., Foucras, G., Gourdy, P., Krust, A., Dupont, S., Chambon, P., Druet, P., Bayard, F. and Guéry, J. C. (2003). Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. The European Journal of Immunology 33: 512-521.
14. Bebo, B. F. Jr., Fyfe-Johnson, A., Adlard, K., Beam, A. G., Vandenbark, A. A.and Offner, H. Low-Dose Estrogen Therapy Ameliorates Experimental Autoimmune Encephalomyelitis in Two Different Inbred Mouse Strains. (2001). The Journal of Immunology. 166: 2080-2089.
15. Korn-Lubetzki, I., Kahana, E., Cooper, G. and Abramsky, O. (1984). Activity of multiple sclerosis during pregnancy and puerperium. Annals of Neurology 16(2): 229-231.
16. Tierney, K. L., Julia, R. H. and Gregory, E. D. (2015). Sexual activity modulates shifts in Th1/Th2 cytokine profile across the menstrual cycle: An observational study. Fertility and Sterility 104 (6): 1513-1521.
17. Amanda E, Anna Maria SR, Michelle P, et al. Effect of pregnancy on disease flares in patients with systemic lupus erythematosus. Ann Rheum Dis. 2018; 77(6): 855-860.