Journal of Depression and Anxiety
ISSN: 2167-1044

Zhang and Wang. J Depress Anxiety 2017, 6:3
DOI: 10.4172/2167-1044.1000271

Research Article Open Access

Disruption of Type I-IFN Signaling Decreases Autoimmune Development and Kidney Damage, But Not Anxiety-Like Behaviors in Lupus NZB Mice

Hua Zhang and Jianping Wang*

Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, USA

Abstract

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder that is accompanied by neuropsychiatric manifestations such as anxiety. Despite undefined etio-pathogenesis for SLE, emerging evidence supports the importance of type I interferon (IFN) in the pathogenesis of autoimmune formation and renal damage both in SLE patients and lupus mice. Linkage mapping identified a quantitative trait locus (QTL) for elevated plus-maze (EPM) performance on the segment of chromosome 4 in lupus-prone New Zealand black (NZB) mice where the type I IFN-α genes are harbored. To determine possible roles of type I IFNs for anxiety-like behaviors in NZB mice, we evaluated the anxiety profile by EPM test in NZB mice with deficiency of type I-IFN receptor (IFNARKO). Consistent with previous observation, disruption of the type I-IFN signaling resulted in a dramatic attenuation in glomerulonephritis, splenomegaly and plasma anti-nuclear antibodies (ANA) in NZB mice. However, blockade of type I-IFN signaling had no effect on performance in the EPM by NZB/IFNARKO mice in comparison to wild type controls. The results support a pathogenic role for type I-IFN in autoimmune development and kidney inflammation. Nonetheless, type I-IFN signaling is not responsible for increased anxiety profile developed in these autoimmune mice.

Keywords: Cytokines; Interferon; Mouse; Neurobiology; Anxiety; Autoimmunity; Cell signaling; CNS lupus

Introduction

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease in humans. With a female/male prevalence ratio of 9 to 1, SLE is characterized by increased proinflammatory cytokines, formation of autoantibodies and immune complexes that affect multiple organ systems [1]. In addition to abnormalities in peripheral tissues and organs, up to 75% of SLE patients develop central nervous system (CNS) manifestations, collectively named neuropsychiatric SLE (NP-SLE) or CNS lupus. Common psychiatric abnormalities include anxiety and cognitive dysfunction [2]. However, the etiology and pathogenesis of SLE remains largely unknown. Nevertheless, a role of type I interferon (IFN) has been suggested in the pathogenesis of SLE by studies both from humans and animal models [3]. For example, gene chip analyses reveal a significantly enhanced transcriptional profile in the cells prepared from SLE patients in response to IFN-α (a major form of type I IFN) [4,5]. Meanwhile, suppression of type I-IFN signaling led to a marked attenuation of autoimmune development and kidney damages in lupus-prone NZB mice [6,7].

Lupus-prone mice such as NZB, NZBWF1 and MLR/lpr exhibit significantly increased anxiety profiles on EPM test and have been used as a model for understanding the etiopathogenesis of anxiety disorders in SLE patients [8,9]. Genome-wide scan and linkage study revealed that the region harboring IFN-α genes on chromosome 4 in NZB mice is linked to the anxiety-like behavior on EPM test in NZB/NZW (New Zealand White) F1 mice [10]. Development of behavioral dysfunctions observed following chronic therapeutic treatment of IFN-α in human patient also suggests a pathogenic activity of this innate immune cytokine for behavioral dysfunction [11]. To directly examine the contribution of type I-IFN signaling to the emotional behavioral dysfunction in lupus mice, the behavioral consequence of ablation of type I-IFN receptor (IFNAR) in female NZB mice was investigated. The impact of type I-IFN receptor deficiency on autoimmune status and tissue damage of NZB mice was also evaluated.

Materials and Methods

Animals and sample collection

Breeding pairs of NZB/IFNARKO and control NZB mice were kindly provided by Drs. Argyrios N. Theofilopoulos and Dwight H. Kono at Scripps Research Institute and were used to produce NZB/IFNARKO and NZB control mice respectively at our laboratory animal research center. Non-autoimmune control NZW mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). In addition to behavior test, mice were sacrificed for blood, spleen and kidney collection at different ages. Spleens were dissected and weighed. Kidneys were collected and paraffin-embedded sections were prepared. Serum was separated for collected blood and stored at -70°C until serologic analysis. Animal handling and experimental procedures were conducted in accordance with the National Institutes of Health (NIH) guidelines for animal care and use and approved by UMKC Institutional Animal Care and Use Committee (IACUC).

Verification of type I-IFN receptor depletion

IFNARKO mice were originally developed at Genentech Inc. (South San Francisco, CA) and were verified by PCR genotyping using DNA extracted from tails [12]. To further confirm functional deficiency of the IFNAR in IFNARKO mice, knockout and wild type mice were treated with a single dose of IFN-α (2×10^5 IU/kg) or vehicle by intraperitoneal injection. Livers were dissected and collected following the treatment. Poly (A+) RNA was extracted by an oligo (dT) cellulose

***Corresponding author:** Jianping Wang, Division of Pharmacology and Toxicology University of Missouri-Kansas City, Kansas City, USA, Tel: + 8162356440; E-mail: wangjp@umkc.edu

Received December 14, 2016; **Accepted** May 12, 2017; **Published** May 16, 2017

Citation: Zhang H, Wang J (2017) Disruption of Type I-IFN Signaling Decreases Autoimmune Development and Kidney Damage, But Not Anxiety-Like Behaviors in Lupus NZB Mice. J Depress Anxiety 6: 271. doi:10.4172/2167-1044.1000271

(Ambion, Austin, TX, USA) method and analyzed for expression of IFN-stimulated genes by RNase protection assay (RPA) as described in our previous studies [13].

Behavioral test

Anxiety profile was analyzed by elevated plus-maze (EPM), a well-characterized paradigm for measuring anxiety in rodents as described in previous studies [14,15]. In brief, the mouse was placed individually in the center platform facing one of the open arms and allowed to freely explore the maze for a total of 5 min. Movements through the maze were detected by equally spaced photocells. Entries into each arm and times spent in each arm were captured via a Dell computer utilizing MotorMonitor software from Kinder Scientific (Poway, CA, USA). The total number of arms entries and total beam breaks represent locomotor activity while the percentage of time spent in open arms and percentage of entries into open arms are indicative of the anxiety profile, independent of the locomotor activity effect [16].

Autoantibodies detection

Anti-nuclear antibodies (ANA) (total immunoglobulin) were detected using an enzyme-linked immunosorbent assay (ELISA) kit from Alpha Diagnostic International (San Antonio, Texas, USA). The assay was performed for collected serum according to manufacturer's instructions. In brief, after color development, the absorbance was measured at 450nm on a 96-well microplate reader (BioTek Instruments, Inc. Winooski, VT, USA). The optical absorbance (OD) value was calculated by subtracting the absorbance of the blank as the measurement of autoantibodies level similar to previous studies [8,17].

Renal histology

Periodic acid-Schiff (PAS) reagents (Sigma-Aldrich, St. Louis, MO, USA) stain basement membrane glycoprotein. PAS staining of kidney sections was used to assess basement membrane thickness and extracellular matrix deposition that are associated with glomerulonephritis [18]. Paraffin-embedded kidney sections (4μm) were stained in Schiff reagent for 15 min. After thorough washing in 0.55% potassium metabisulfite and tap water, the sections were counterstained in Harris' hematoxylin. The stained sections were viewed under an Olympus BX60F5 photomicroscope (Olympus, Optical Co Ltd, Japan) and images were captured by an attached digital microscope camera (MIS, Inc. Villa Park, IL, USA).

Statistical analysis

Results are expressed as mean ± standard error of means (SEM). Two-tailed unpaired Student's *t*-test was conducted for data analysis. $p \leq 0.05$ was considered as statistically significant difference for all comparisons.

Results

The genotypes of NZB and NZB/IFNARKO mice were verified by PCR assay using extracted tail DNA. Since type I-IFN receptor is required for IFN-α action, we decided to analyze the expression of IFN-regulated genes following a high dose of IFN-α treatment as a functional assay to confirm the deletion of IFNAR in NZB/IFNARKO mice. These include several prototypic IFN-stimulated genes, dsRNA-dependent protein kinase R (PKR), ubiquitin-specific proteinase 18 (USP18), signal transducer and activator of transcription (STAT1), IFN-induced 15 kDa protein (ISG15), guanylate-binding protein 3 (GBP3) and IFN-induced 10 kDa protein (IP10 or CXCL10) as described in our previous studies [13]. As expected, IFN-α treatment induced a profound stimulation of the expression of these genes in wild type NZB mice (Figure 1). In contrast to NZB control, IFN-α challenge resulted in no stimulation of IFN-stimulated gene expression in NZB/IFNARKO mice because of the loss of functional type I-IFN receptor in these mice.

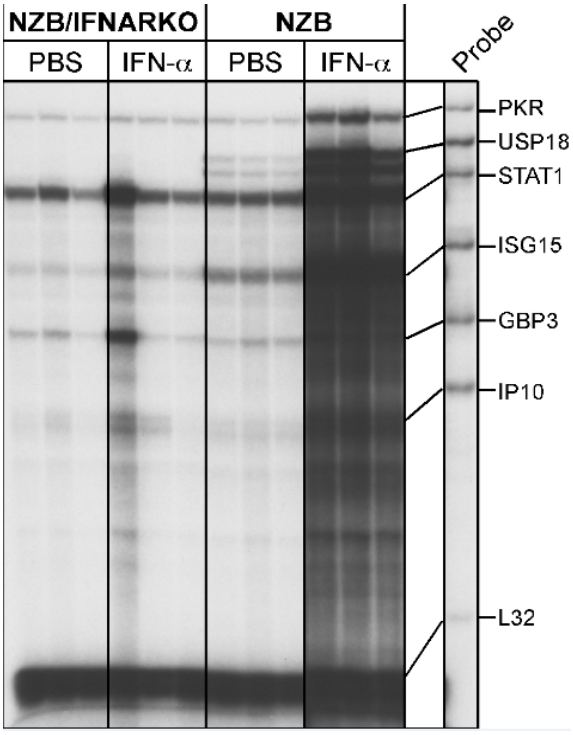

Figure 1: Verification of IFNARKO mice. NZB/IFNARKO and wild type NZB mice were injected intra-peritoneally with a single dose of mouse IFN-α (2×10^5 IU/kg) or vehicle, and livers were collected 4 hours later for poly (A^+) RNA preparation. One μg of poly (A^+) RNA were analyzed by RPA. Each lane represents an individual animal.

Similar to previous observation [8], a significant difference in both percentage of time in open arms and percentage of entries into open arms, two measures of anxiety-like behavior by elevated plus-maze (EPM) was found between autoimmune NZB and control NZW mice at 12 weeks of age (Figure 2A left panel). Compared to non-autoimmune NZW mice, NZB mice showed a dramatic decrease in these two measurements by 71% ($p<0.01$) and 45% ($p<0.01$) respectively, indicating increased anxiety profile in lupus mice. Nonetheless, there were no significant differences between the two groups of mice in measurements of the general locomotor activity, the total number of arms entries and total number of beam breaks (Figure 2A right panel).

To our surprise, in comparison with the control NZB females, NZB/IFNARKO mice did not exhibit any alterations in their performance on elevated-plus maze (Figure 2B left panel). The anxiety level measured by the percentage of time spent in open arms and the percentage of entries into open arms were not changed in NZB/IFNARKO versus control NZB mice. The results reveal that blockade of type I-IFN signaling has no impact on the emotional behavioral abnormalities that are developed in NZB female mice. Additionally, with no functional type IFN receptor, NZB/IFNARKO mice also did not alter locomotor activities measured by total aim entries and total beam breaks in EPM test (Figure 2B right panel).

To confirm the critical effect of type I-IFN on autoimmune development and kidney damage reported in previous studies [6,7],

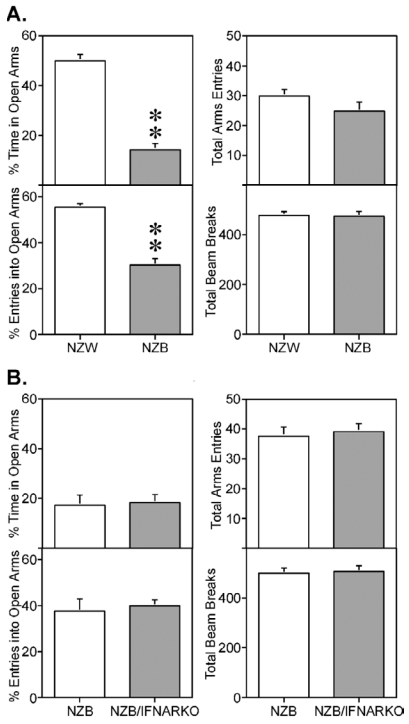

Figure 2: Disruption of type I IFN signaling does not improve the performance of lupus mice in the elevated plus-maze (EPM) test. Mice were tested on the EPM for 5 minutes at 12 weeks of age and the percentage of time spent in open arms, the percentage of the entries into open arms, the total number of arms entries and the total number of beam breaks was compared between two groups. A. Female NZW and NZB mice (n = 10, **: $p < 0.01$); B. Female NZB and NZB/IFNARKO mice (n = 8).

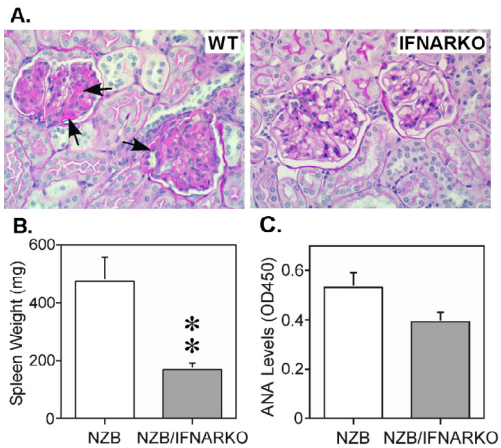

Figure 3: Blockade of type I IFN signaling attenuates autoimmune development and kidney damage in lupus mice. A. Representative kidney sections from 9-month old NZB and NZB/IFNARKO mice stained with PAS and counterstained with hematoxylin. The arrows reveal PAS stained thickened glomerular capillaries (Magnification: ×400); B. Spleen weights of NZB and NZB/IFNARKO mice at 9 months of age (n = 5-6; **: $p < 0.01$); C. Serum anti-nuclear autoantibodies (ANA) levels analyzed by ELISA at 14 weeks of age (n = 8).

ELISA of anti-nuclear antibody (ANA) and periodic-acid Schiff (PAS) staining of kidney sections were performed to assess autoimmune response and basement membrane thickness, as well as extracellular matrix deposition associated with glomerulonephritis respectively. As shown in (Figure 3), thickened glomerular capillaries, thickening in the basement membrane and severe extracellular matrix deposition in glomeruli were substantially reduced in NZB/IFNARKO mice in comparison with the wild type NZB mice (Figure 3A). Autoimmune hemolytic anemia is another major clinical manifestation in NZB mice that is characterized by splenomegaly [19]. We then compared the differences in spleen weight between these two lines of mice. As expected, blockade of type I-IFN signaling by knocking out its receptor dramatically reduced splenomegaly, from 480 mg in NZB mice to 180 mg in NZB/IFNARKO mice close to those from non-autoimmune control animals (Figure 3B). Serologic analysis revealed a decrease in serum ANA level by 26% in NZB/IFNARKO mice compared to NZB control mice ($p = 0.07$) (Figure 3C). Together, the results confirmed an importance of type I-IFN signaling in the development of autoimmunity and kidney damage [3,6].

Discussion

The present study confirms that disruption of type I-IFN signaling reduces autoimmune development and renal damage in autoimmune NZB mice. Significant attenuation of the autoimmune response and tissue inflammation in NZB/IFNARKO mice is detected by amelioration of glomerulonephritis, blockade of splenomegaly and decreased levels of serum ANA. Such observation provides further support for the importance of type I-IFN in the pathogenesis of autoimmune development and kidney injury [3]. Nevertheless, despite the reports of type I interferon for autoimmune-induced psychiatric abnormality developed in SLE patients [20] and lupus-prone mice [10], inhibition of type I-IFN signaling by knocking out its receptor does not change the performance of these mice on elevated-plus maze. NZB/IFNARKO mice exhibit an anxiety profile that is similar to those displayed in control NZB mice. The finding suggests that type I-IFN signaling is not required for the increased anxiety profile developed in these autoimmune mice. Nonetheless, previous studies reported that lupus-prone mice develop behavior changes in parallel with the autoimmune process, suggesting that behavioral dysfunctions are the consequence of autoimmune disease [8,9].

The importance of type I-IFN for anxiety-like behavior was detected in autoimmune NZB/NZW F1 mice [10]. Such findings are supported by elevated anxiety profile following chronic IFN-α treatments in humans [21], mice [10] and rats [22]. The reasoning for the observed discrepancy for the role of type I-IFN signaling in behavioral deficits in lupus-prone mice is unknown. However, a number of factors may contribute to the opposite observations. These include 1) different anxiety paradigms were used in different studies [22]; 2) in addition to type I-IFN, other innate immune mediators such as tumor necrosis factor-α and interleukin-1, which can also contribute to development of anxiety in autoimmune lupus mice [21]. On the other hand, conventional transgenic mice with brain-targeted expression of transgene may change programmed brain development that results in behavioral alterations. In this regard, further investigation is warranted in order to confirm the etiopathogenic role for type I-IFN in autoimmune lupus as well as psychiatric disorders.

Recent clinical investigations have detected a possible relationship between autoantibodies including anti-ribosomal P (anti-P), anti-phospholipid or anti-NR2 glutamate receptor antibodies and neuropsychiatric manifestations of SLE in humans [23-25]. There was a report that the IgG prepared from lupus patient's cross-reacts with dsDNA and the N-methyl-aspartate receptor (NMDA) receptor [26]. The antibodies, when injected into the mice, caused neuronal apoptosis and elicited learning deficits [27,28]. Treatment of pregnant mice with the antibody against the NR2-specific NMDA receptor can disrupt the

development of the neocortex in the fetal brain and led to subsequent cognitive deficits in adult offspring [28]. Various autoantibodies against host molecules may cross the disrupted blood-brain barrier due to the inflammatory process or the uteroplacental barrier and eventually lead to the functional and/or structural damage that accounts for the CNS manifestations of SLE [27,29].

It should be pointed out that other than autoantibodies against nuclear antigen (ANA) and double-stranded DNA (anti-dsDNA) detected in autoimmune lupus mice [19], it remains largely unknown whether any autoantibodies specific against neural cells and nervous tissue is developed in SLE-prone NZB mice. Therefore, future development of similar assays to detect suspected autoantibodies against neurons or/and glia cells in mice will shed the light on the contribution of specific autoantibodies to the anxiety-like behavior manifested in these autoimmune mice.

Conclusion

In summary, our findings from this study demonstrate while type I-IFN signaling is critical for autoimmune formation and inflammatory damage in lupus-prone mice, the unchanged anxiety-like behavior detected by elevated plus maze in NZB/IFNARKO mice indicates a type I IFN-independent mechanism for behavioral dysfunction in these autoimmune mice.

Conflict of Interest Statement

All authors declare that there are no conflicts of interest.

Acknowledgements

We thank Drs. Theofilopoulos and Kono (Scripps Research Institute), and Genentech Inc. for providing NZB/IFNARKO mice. This study was supported in part by NIH Grants MH 69524, and the University Missouri Research Board (UMRB) grant to J.W.

References

1. Tutuncu ZN, Kalunian KC (2007) The definition and classification of systemic lupus erythematosus. In: Dubois' Lupus Erythematosus. Wallace DJ, & Hahn BH (eds) Lippincott Williams & Wilkin, Philadelphia, pp: 16-20.
2. Meszaros ZS, Perl A, Faraone SV, Andras P (2012) Psychiatric symptoms in systemic lupus erythematosus: A systematic review. The Journal of clinical psychiatry 73: 993-1001.
3. Crow MK (2014) Type I interferon in the pathogenesis of lupus. Journal of Immunology 192: 5459-5468.
4. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, et al. (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci USA 100: 2610-2615.
5. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, et al. (2003) Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 197: 711-723.
6. Santiago-Raber ML, Baccala R, Haraldsson KM, Choubey D, Stewart TA, et al. (2003) Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J Exp Med 197: 777-788.
7. Jorgensen TN, Roper E, Thurman JM, Marrack P, Kotzin BL (2007) Type I interferon signaling is involved in the spontaneous development of lupus-like disease in B6.Nba2 and (B6.Nba2 x NZW)F(1) mice. Genes Immun 8: 653-662.
8. Schrott LM, Crnic LS (1996) Anxiety behavior, exploratory behavior, and activity in NZB x NZW F1 hybrid mice: Role of genotype and autoimmune disease progression. Brain Behav Immun 10: 260-274.
9. Sakic B, Szechtman H, Denburg JA (1997) Neurobehavioral alterations in autoimmune mice. Neurosci Biobehav Rev 21: 327-340.
10. Nakamura K, Xiu Y, Ohtsuji M, Sugita G, Abe M, et al. (2003) Genetic dissection of anxiety in autoimmune disease. Hum Mol Genet 12: 1079-1086.
11. Schaefer M, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, et al. (2002) Interferon alpha (IFNalpha) and psychiatric syndromes: a review. Prog Neuropsychopharmacol Biol Psychiatry 26: 731-746.
12. Muller U, Steinhoff U, Reis LFL, Hemmi S, Pavlovic J, et al. (1994) Functional role of type I and type II interferons in antiviral defense. Science 264: 1918-1921.
13. Wang J, Campbell IL, Zhang H (2008) Systemic interferon-α regulates interferon-stimulated genes in the central nervous system. Mol Psychiatry 13: 293-301.
14. Zhang H, Tian Z, Wang J (2010) Behavioral evaluation of transgenic mice with CNS expression of IFN-α by elevated plus-maze and Porsolt swim test. Neurosci Lett 479: 287-291.
15. Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 92: 180-185.
16. Hogg S (1996) A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 54: 21-30.
17. Havarinasab S, Hultman P (2006) Alteration of the spontaneous systemic autoimmune disease in (NZB x NZW) F1 mice by treatment with thimerosal (ethyl mercury). Toxicol Appl Pharmacol 214: 43-54.
18. Grande JP, Balow JE (1998) Renal biopsy in lupus nephritis. Lupus 7: 611-617.
19. Andrews BS, Eisenberg RA, Theofilopoulos AN, Izui S, Wilson CB, et al. (1978) Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. J Exp Med 148: 1198-1215.
20. Shiozawa S, Kuroki Y, Kim M, Hirohata S, Ogino T (1992) Interferon-alpha in lupus psychosis. Arthritis Rheum 35: 417-422.
21. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, et al. (2001) Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 344: 961-966.
22. Myint AM, O'Mahony S, Kubera M, Kim YK, Kenny C, et al. (2007) Role of paroxetine in interferon-alpha-induced immune and behavioural changes in male Wistar rats. J Psychopharmacol 21: 843-850.
23. Hanly JG, Urowitz MB, Siannis F, Farewell V, Gordon C, et al. (2008) Autoantibodies and neuropsychiatric events at the time of systemic lupus erythematosus diagnosis: results from an international inception cohort study. Arthritis Rheum 58: 843-853.
24. Mikdashi J, Handwerger B (2004) Predictors of neuropsychiatric damage in systemic lupus erythematosus: data from the Maryland lupus cohort. Rheumatology (Oxford) 43: 1555-1560.
25. Steup-Beekman G, Steens S, Van Buchem M, Huizinga T (2007) Anti-NMDA receptor autoantibodies in patients with systemic lupus erythematosus and their first-degree relatives. Lupus 16: 329-334.
26. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, et al. (2001) A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. Nat Med 7: 1189-1193.
27. Huerta PT, Kowal C, De Giorgio LA, Volpe BT, Diamond B (2006) Immunity and behavior: Antibodies alter emotion. Proc Natl Acad Sci U S A 103: 678-683.
28. Lee JY, Huerta PT, Zhang J, Kowal C, Bertini E, et al. (2009) Neurotoxic autoantibodies mediate congenital cortical impairment of offspring in maternal lupus. Nat Med 15: 91-96.
29. Kowal C, DeGiorgio LA, Nakaoka T, Hetherington H, Huerta PT, et al. (2004) Cognition and immunity; antibody impairs memory. Immunity 21: 179-188.