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

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Abstract

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder that is accompanied by neuropsychiatric manifestations such as anxiety. Despite undefined eti-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, NZBW/F1 and MRL/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 × 10⁸ 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
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 a 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.

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 I-IFN receptor, NZB/IFNARKO mice also did not alter 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].
As shown in (Figure 3), thickened glomerular capillaries, thickening matrix deposition associated with glomerulonephritis respectively. ELISA of anti-nuclear antibody (ANA) and periodic-acid Schiff (PAS) staining of kidney sections were performed to assess autoimmune development and kidney damage. 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).


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

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 mouse 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].
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 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 (Scirpp 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.

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