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Development of Autoimmunity in IL-14α-Transgenic Mice

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Multiple genetic loci contribute to the development of systemic lupus erythematosus (SLE). In murine models for SLE, various genes on chromosome four have been implicated. IL-14 is a cytokine originally identified as a B cell growth factor. The il14 gene is located on chromosome 4. IL-14α is a cytokine encoded by the plus strand of the IL-14 gene using exons 3–10. The expression of IL-14α is increased in (NZB × NZW)F1 mice. In this study, we produced IL-14α-transgenic mice to study the role of IL-14α in the development of autoimmunity. At age 3–9 mo, IL-14α-transgenic mice demonstrate increased numbers of B1 cells in the peritoneum, increased serum IgM, IgG, and IgG2a and show enhanced responses to T-dependent and T-independent Ags compared with littermate controls. At age 9–17 mo, IL-14α-transgenic mice develop autoantibodies, slaladenitis, as in Sjögren’s syndrome, and immune complex-mediated nephritis, as in World Health Organization class II SLE nephritis. Between the ages 14–18 mo, 95% of IL-14α-transgenic mice developed CD5+ B cell lymphomas, consistent with the lymphomas seen in elderly patients with Sjögren’s syndrome and SLE. These data support a role for IL-14α in the development of both autoimmunity and lymphomagenesis. These studies may provide a genetic link between these often related disorders.

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3 Abbreviations used in this paper: SLE, systemic lupus erythematosus; NZB, New Zealand Black; NZW, New Zealand White; ANA, anti-nuclear Ab; sIg, surface Ig; FISH, fluorescence in situ hybridization; NP, nitrophenyl.

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hypergammaglobulinemia and IgM anti-cardiolipin Abs but only rarely IgG ANA and anti-DNA Abs. IL-14α-transgenic mice also show enhanced responses to T-dependent and T-independent Ags, sialadenitis, and immune complex-mediated nephritis with deposition of IgM in their glomeruli. The IL-14α transgene induced a phenotype that is very similar to SLE and Sjögren’s syndrome. In addition, most aged IL-14α-transgenic mice develop a CDS B cell lymphoma similar to the tumors seen in elderly patients with SLE and Sjögren’s syndrome (37–39).

Materials and Methods

Mice

B6 mice were obtained from The Jackson Laboratory and housed in the Laboratory Animal Facility at State University of New York (SUNY; Buffalo, NY) in accordance with institutional guidelines. IL-14α-transgenic mice were made by the Gene Targeting and Transgenic Facility at Roswell Park Cancer Institute (Buffalo, NY) and maintained in the Laboratory Animal Facility at SUNY Buffalo.

Production of transgenic mice

To produce the transgenic mice, the pEpmSR vector expressing IL-14α was constructed as previously described (40). In brief, the cDNA for human IL-14α (amplified by RT-PCR from total RNA purified from PHA stimulated-Namalva cells with primers that also included the necessary polylinker sites and a hemagglutinin tag) was cloned into pBluescript using the endonuclease sites NcoI and BglII. The cDNA for hemagglutinin-IL-14α was then inserted into the pEpmSR polylinker sites by using the endonuclease sites HindIII and SST1. The completed vectors were transfected into DHSα cells that were expanded in Luria-Bertani medium. The transgenic vectors were then isolated using the Lysozyme-Trition plasmid prep kit (Qiagen) and further purified by CsCl centrifugation. The purified vectors were cut with NotI and the vector DNA containing the human IL-14α cDNA was purified using the QiAquick gel extraction kit (Qiagen).

Purified vectors were then injected into C3H oocytes fertilized with B6 sperm and placed into B6 pseudopregnant females. The presence of the transgenic vector in the offspring was determined by PCR of tail DNA purified by the DNeasy Tissue kit (Qiagen) using the vector-specific primers: forward-AGGCCTGTACGGAATGTTACTTC and reverse-CAGCCTGACCTGGAGGATGAATT. Positive offspring were then crossed to B6 partners. The purified vectors were cut with NotI and the vector DNA containing the human IL-14α cDNA was purified using the QiAquick gel extraction kit (Qiagen).

The transgenic vector in the offspring was determined by PCR of tail DNA purified by the DNeasy Tissue kit (Qiagen) using the vector-specific primers: forward-AGGCCTGTACGGAATGTTACTTC and reverse-CAGCCTGACCTGGAGGATGAATT. Positive offspring were then crossed to B6 partners. The purified vectors were cut with NotI and the vector DNA containing the human IL-14α cDNA was purified using the QiAquick gel extraction kit (Qiagen).

Fluorescence in situ hybridization (FISH)

For the analysis of the chromosomal localization of the IL-14α transgene, metaphase chromosome spreads were prepared from cultured fibroblasts derived from the spleens of IL-14α-transgenic mice using standard hypotonic and air-drying procedures (41). The probe was derived from a 5.3-kb region of chromosome 1 including the endonuclease sites HindIII and SST1. The probe was then labeled by in vitro transcription using T7 phage RNA polymerase with biotin label UTP according to the manufacturer's instructions (MAXI Script In Vitro Transcription kit; Ambion). The probes were purified using 6% Tris-borate/EDTA/urea gels at 58°C for 1 min and then stored at 4°C. The primers used for the actin control were: forward-5'-GGGGCCCGGACCA and reverse-5'-CTCCTTAATGTCACGGCAGATTTC. For the IL-14α mRNA, the primers were forward-5'-GCCACAGAAGAAGAACGCAA and reverse-5'-GACTTCCACCCCTTGTCCTTGG.

RT-PCR products were separated on 1% agarose gels and visualized by UV light after incorporation of ethidium bromide, as described previously (32).

Northern blot analysis for murine IL-14α

The DNA probes for the Northern blots were prepared as follows: RT-PCR using the forward primers-5'-ATGAGGACGACCA and reverse primers-5'-GGGCACACTGGAGAGTGAATT. Positive offspring were then crossed to B6 partners. The purified vectors were cut with NotI and the vector DNA containing the human IL-14α cDNA was purified using the QiAquick gel extraction kit (Qiagen).

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Mouse cell separation

Dynameics mouse pan B (B220; Invitrogen Life Technologies) was used to isolate mouse B220-positive and -negative cells from spleen following the manufacturer’s instruction.

Total RNA purification

Dependent on the source of samples, two different methods were used to collect total RNA. TRIzol purified total RNA from the whole spleen of mice, according to the manufacturer’s instructions (Invitrogen Life Technologies). Total RNA from cells harvested from the spleens of mice was purified with the QiAamp RNA Blood mini kit according to the manufacturer’s instructions (Qiagen).

Semiquantitative RT-PCR

cDNA was first produced from total RNA using the SuperScript first-strand synthesis system for RT-PCR according to the manufacturer’s instructions (Invitrogen Life Technologies), then amplified with specific primers under the following conditions for PCR: 3 min of denaturation at 94°C, followed by 35 cycles of 94°C for 1 min, extension at 72°C for 1 min and denaturation at 94°C for 1 min. At the end of the 35 cycles, the reactions were maintained at 72°C for 5 min and then stored at 4°C. The primers used for the actin control were: forward-5’-GGGGCCCGGACCA and reverse-5’-CTCCTTAATGTCACGGCAGATTTC. For the IL-14α mRNA, the primers were forward-5’-GCCACAGAAGAAGAACGCAA and reverse-5’-GACTTCCACCCCTTGTCCTTGG.

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anti-murine IgM (Sigma-Aldrich). Analysis was then performed using fluorescence microscopy, as described (45).

Flow cytometry

Cells collected from peritoneal cavity of the mice, spleens, or tumor tissue of the mice were stained with panels composite with anti-mouse Ab CD19-allophycocyanin, CD138-PE, CD21-FITC, CD5-PE-Cy5, CD23-PE, IgD-FITC (BD Pharmingen), CD38-PE-Cy5 (eBioscience), and IgM-PE (Abcam) and analyzed on a BD Biosciences FACSCalibur machine and Winlist software.

Immunohistochemistry for CD5 and B220

Tumor tissues from IL-14/H9251-transgenic mice were collected and fixed in 10% formalin then embedded in paraffin. Thin sections were prepared on slides and the slides stained with the Histomouse-Max kit (Invitrogen Life Technologies) as instructed by the manufacturer. Anti-mouse CD5 and B220Abs were purchased from BD Pharmingen.

Ig gene rearrangements

Clonal B cell determination of mouse tumor was performed by high-fidelity PCR using 0.1 mg of DNA extracted from normal B6J mouse liver, spleen, and transgenic mouse tumors using the DNeasy Tissue kit (Qia-gen). The primers for Ig gene rearrangement in the lymphomas from the IL-14α-transgenic mice were: DHL, 5'-GGAATTCGATTTTTGTG TTTTTGCTACTCTAGTG; J3, 5'-GTTCTAGATTTCCAAAGAGTTCCGATAGCCCTG; and DHR, 5'-TTTGGTMTGATATAKCGCTGAC; J3, 5'-GTTCTAGATTTCCAAAGAGTTCCGATAGCCCTG. PCR was performed as described (46) with the following protocol: incubation 95°C for 5 min then 95°C 1 min, 55°C 1 min, and 72°C 2 min for 35 cycles, followed by extension at 72°C for 10 min. The PCR products were analyzed on 1.2% agarose gel electrophoresis.

Oversight

The animal studies were all approved by the Institutional Animal Care and Use Committee (SUNY Buffalo School of Medicine and Biomedical Sciences).

Results

The transcript we have designated IL-14/H9251 is generated from the plus strand of the il14 gene using exons 3–10. The predicted amino acid sequence encoded by this transcript predicts a 47.4-kDa protein with >90% amino acid sequence identity between human and mouse (Fig. 1). This transcript is expressed normally in activated T lymphocytes, a subpopulation of activated B lymphocytes (see Fig. 3C) and in follicular dendritic cells (our unpublished data). Because the il14 gene was mapped to a potential lupus susceptibility locus in both human and mouse (34, 47), we first examined the expression of IL-14α in the splenic lymphocytes of NZB, NZW, and (NZB × NZW)F1 mice, a well-established animal model for SLE. As demonstrated in Fig. 2 by Northern blot analysis, the IL-14α mRNA is expressed in 8-mo-old female NZW and (NZB × NZW)F1 mice, a well-established animal model for SLE. As demonstrated in Fig. 2 by Northern blot analysis, the IL-14α mRNA is expressed in 8-mo-old female NZW and (NZB × NZW)F1 mice, a well-established animal model for SLE. As demonstrated in Fig. 2 by Northern blot analysis, the IL-14α mRNA is expressed in 8-mo-old female NZW and (NZB × NZW)F1 mice, a well-established animal model for SLE.

FIGURE 1. The protein sequences were aligned with Clustal X and then box-shaded via the BOXSHADE server at (www.ch.embnet.org/software/BOX_form.html). Dark shaded residues are identical; light shaded residues are similar; unshaded residues differ.

FIGURE 2. Splenic lymphocytes were obtained from 8-mo-old female mice and total RNA was prepared as described in Materials and Methods. Northern blots were performed using cDNA probe based on exons 2 and 3 of IL-14, as described in Materials and Methods. RNA was made from the spleens of individual mice. The mouse strains are B6, NZB, NZW, and (NZB × NZW)F1 (F1). The RNA bands were visualized with the BioDetect kit (Ambion). The IL-14α signal was identified as a 4.1-kb band whereas actin was identified as a 2.1-kb band.
evaluation of the ability of IL-14α to induce autoimmunity in transgenic mice.

**Generation of IL-14α-transgenic mice**

To understand physiological roles for IL-14α in vivo, we generated transgenic mice using pEμSR (40). pEμSR vector leads to gene expression predominantly in the B cell compartment. Three lines of transgenic mice were initially derived and studied for various properties including serum IgG and responses to vaccinations with T-dependent and T-independent Ags. All three lines behaved similarly in these assays, so one line was selected for further backcrosses to enhance the purity of the B6 background and for further study. This transgenic line of mice contained the pEuSR-IL-14α transgene predominantly on murine chromosome 18 (Fig. 3A), and expressed IL-14α mRNA predominantly in the B lymphocyte compartment (Fig. 3B). The level of expression of the transgene in these mice was similar to the level of IL-14α expression seen in T cells activated with PHA or B cells activated with LPS from normal B6 mice or from splenic lymphocytes of 8-mo-old female (NZB × NZW)F1 splenocytes (Fig. 3C). Chromosome 18 does not contain genes that are known to influence either autoimmunity or lymphomagenesis. The IL-14α protein was detectable in the sera of these mice using rabbit polyclonal antisera against human IL-14α (data not shown). The IL-14α-transgenic mice used in this study were backcrossed for eight generations with B6 mice to minimize the presence of non-B6 genes. Both male and female mice were used in all the studies. No qualitative differences were found between males and females, although females tended to develop autoimmunity somewhat earlier and more severely than males.

**Serum studies in IL-14α-transgenic mice**

Serum was collected from IL-14α-transgenic mice at different ages to evaluate production of IgG and autoantibodies. The IL-14α-transgenic mice develop hypergammaglobulinemia by 6 mo of age involving both IgG and IgM that becomes more exaggerated as they get older (Fig. 4A). At 9 mo of age, the IL-14α-transgenic mice had a statistically significant increase in IgA compared with the littermate controls (p < 0.0001). They had a more prominent elevation in IgG2a (p < 0.0001), but not in the other IgG subclasses (Fig. 4B). Because IgG2a is usually induced by IFN, we also examined levels of IFN-α, IFN-β, and IFN-γ in the sera of these mice. No IFN-β or IFN-γ was detected. A significant amount of IFN-α was noted in the sera of the IL-14α-transgenic mice, but not in the littermate controls at 10–12 mo of age (Fig. 4C). We next examined in the sera of these mice the presence of autoantibodies often associated with SLE and Sjögren’s syndrome, IgG ANA, anti-dsDNA, anti-chromatin, anti-Ro, anti-La, anti-Sm, and anti-nRNP (48, 49). Forty mice were included in these studies. Although some of the mice had high titers of one or more of these Abs, many of the IL-14α-transgenic mice did not express any of them. The only autoantibody identified in the sera of all of the IL-14α-transgenic mice was IgM anti-cardiolipin. The titer of this autoantibody increased as the mice aged and was statistically significantly different from the normal controls at all the ages tested (a representative study with six mice in each group: 6 mo, p = 0.0034; 9 mo, p = 0.0084; 12 mo, p < 0.0001) (Fig. 4D).

**Vaccination studies and B cell subpopulations in IL-14α-transgenic mice**

Because previous studies had suggested that IL-14 may influence memory B cell function (33), we vaccinated the IL-14α-transgenic mice with both T-independent and T-dependent Ags and measured their Ag-specific Ab responses. As demonstrated in Fig. 5, IL-14α-transgenic mice demonstrated increased IgG anti-NP responses to the T-dependent Ag NP-OVA compared with littermate controls. When the T-independent Ag NP-Ficoll was used IL-14α-transgenic mice demonstrated increased IgM anti-NP responses (Fig. 5). These studies suggested that several B cell subpopulations

**FIGURE 3.** A, FISH was performed on chromosomal preparations from the fibroblasts of the spleens of IL-14α-transgenic mice using the pEμSR-IL-14α vector as a probe, as described in Materials and Methods. Positive immunofluorescence (green) is noted on chromosome 18. B, Splenic lymphocytes from IL-14α-transgenic mice were isolated and separated with anti-B220 magnetic beads into B220-positive and -negative subpopulations. The RNA was isolated from each of these subpopulations and evaluated by RT-PCR for expression of IL-14α and actin, as described in Materials and Methods. Lane 1, Female IL-14α-transgenic, B220− cells; lane 2, female IL-14α-transgenic, B220+ cells; lane 3, male IL-14α-transgenic, B220+ cells; lane 4, male IL-14α-transgenic, B220− cells; lane 5, nontemplate control. C, Splenic lymphocytes from B6 mice were first isolated and separated with anti-B220 magnetic beads into B220-positive and -negative subpopulations. Then B220-positive cells were placed in culture for 18 h either in medium alone (RPMI 1640/5% FBS) or medium plus LPS (100 μg/ml). B220-negative cells were placed in culture for 18 h with medium alone or medium plus PHA (10 μg/ml). Cells were then harvested, RNA was prepared, and RT-PCR was performed for IL-14α and actin as described in Materials and Methods. Lane 1, C57; lane 2, C57, B220− cells; lane 3, C57, B220+ cells stimulated with LPS; lane 4, C57, B220− cells; lane 5, C57, B220+ cells stimulated with PHA; lane 6, NZBW F1; lane 7, IL-14α-transgenic; lane 8, nontemplate control.
FIGURE 4. A, Sera were obtained from IL-14α-transgenic mice and littermate controls at 3, 6, and 12 mo of age. ELISA was used to determine the levels of IgM and IgG as outlined in Materials and Methods. Data shown are the mean and SEM for the four mice in each group at each time point. The differences between the levels of IgM in the sera of the IL-14α-transgenic mice and littermate controls were significant at all the time points studied (3 mo, $p = 0.0022$; 6 mo, $p = 0.015$, and 12 mo, $p = 0.0021$) as were the levels of IgG (3 mo, $p = 0.04$, 6 mo, $p = 0.0031$, and 12 mo, $p < 0.0001$). B, Sera were obtained from IL-14α-transgenic mice and littermate controls at 9 mo of age. ELISAs were used to determine the levels of IgA, IgE, and the IgG subclasses. Data shown are the individual values for the six mice studied in each group. The only statistically significant differences between the IL-14α-transgenic mice and littermate controls were IgA ($p < 0.0001$) and IgG 2a ($p < 0.0001$). C, ELISA was used to determine the levels of IFN-α in the sera of IL-14α-transgenic mice and littermate controls at the times indicated. Data shown are the mean and SEM for four mice studied in each group. (Figure legend continues)
might be increased either in number or function in the IL-14α-transgenic mice. Therefore, we examined subpopulations of B lymphocytes in the spleen and peritoneal cavities of IL-14α-transgenic mice compared with littermate controls at 12 mo of age. Flow cytometry studies were performed using groups of six IL-14α-transgenic mice and littermate control mice and Abs to CD5, CD19, CD21, CD38, CD138, IgM, and IgD. Several interesting findings are summarized in Table I. In the peritoneal washes, there was a statistically significant increase in the percentage of CD5+, CD19+, IgM+, IgG+, and IgD+ B1 cells in the IL-14α-transgenic mice compared with the littermate controls (14.91 ± 3.0 vs 4.9 ± 0.65; p < 0.0001). In the spleen, the IL-14α-transgenic mice compared with the littermate controls had an increased percentage of total CD19+ B cells (33.69 ± 4.27 vs 18.42 ± 1.6; p < 0.0001), CD19+, CD21+, IgM+ marginal zone B cells (11.78 ± 3.35 vs 1.76 ± 0.49; p < 0.0001), and CD19+, CD38+, slgDlow germinal center B cells (9.51 ± 1.65 vs 5.30 ± 1.15; p = 0.0004). There was a statistically significant increase in CD19+, CD138+ plasma cells in the IL-14α-transgenic mice, although the numbers were small (1.45 ± 0.43 vs 0.48 ± 0.1; p = 0.0003). The absolute numbers of lymphocytes in the spleens of IL-14α-transgenic mice were not significantly different from those in littermate controls (for six mice in each group: IL-14α-transgenic −3.23 ± 0.49 × 107; littermate controls −2.25 ± 0.53 × 107; p = 0.2).

Histological demonstration of autoimmunity

The IL-14α-transgenic mice live a normal lifespan. To study the development of organ injury, mice were autopsied at different ages. By 8 mo of age, most of the IL-14α-transgenic mice (21 of 25 studied) have mild proteinuria, but never develop renal dysfunction. Further studies were performed on 18 mice at 10 mo of age. Histological evaluation of their kidneys revealed only a mild increase in mesangial cells (Fig. 6A). Immunohistochemistry revealed deposition of IgM in the glomeruli (Fig. 6B). Weak deposition of IgG and complement was also noted (data not shown). Interestingly, IgM deposition was also noted in blood vessels. The majority of IL-14α-transgenic mice (39 of 40 mice studied), but not the littermate controls, also developed lymphocytic infiltration of their salivary glands that was age dependent (Fig. 6C). These findings are consistent with SLE and secondary Sjögren’s syndrome.

Lymphoma in IL-14α-transgenic mice

When aged, the IL-14α-transgenic mice all develop mild splenomegaly and lymphoid hyperplasia but not clinically detectable lymph node swelling. Autopsies done on IL-14α-transgenic mice between the ages of 12–20 mo revealed lymphoma in 96% of 40 mice studied. A few mice had lymphoma restricted to the spleen, but the majority had lymphoma in the liver and gastrointestinal tract or lung (Fig. 7). The lymphoma had the histological features of a large B cell lymphoma, expressed CD5 and CD19 but not CD21 and variably CD23. It contained Ig gene rearrangements suggesting a B cell tumor of monoclonal origin (Fig. 7). This development of B cell lymphomas with this pattern of organ involvement is consistent with what is seen in patients with SLE and Sjögren’s disease (38, 39, 50).

Discussion

This study demonstrates that IL-14α is a highly conserved protein in humans and mice. Its expression is increased in an animal model for SLE, the (NZB × NZW) F1 mouse. With enhanced expression of IL-14α, transgenic mice developed multiple physiological changes similar to those observed in patients with SLE and Sjögren’s syndrome. These include hypergammaglobulinemia, involving IgG, IgA, and IgM autoantibodies, deposition of Igs in the kidney and infiltration of the salivary glands with lymphocytes. In addition, the IL-14α-transgenic mice demonstrate increased numbers of B1 cells in their peritoneal cavities and enhanced responses to vaccinations with both T-dependent and T-independent Ags, features
that have been ascribed to the sle2 locus of the NZM mouse (28, 51). Aged IL-14α-transgenic mice develop B cell malignancies that are similar to those seen in patients with SLE and Sjögren’s syndrome (37, 39, 48, 52, 53).

The il14 gene is located near lck, a region that has been associated with various lupus susceptibility as well as lupus suppressor genes (28). A syntenic region on human chromosome 1 was also identified as a lupus susceptibility locus in certain human studies (34, 35). The sle2 locus is on chromosome 4 (28). The dominant phenotype ascribed to this locus is increased B1 cells in the peritoneal cavity (28, 51, 54). Other features ascribed to this locus that are also identified in the IL-14α-transgenic mice are increased serum IgM, enhanced responses to T-dependent and T-independent Ags, and production of anti-cardiolipin Abs (29, 30, 55). Various studies have demonstrated, however, that the effects of sle2 in generating these phenotypic changes are contributed from NZB, rather than NZW (56). The expression of IL-14α is noted in NZW but not NZB mice (Fig. 2). NZW contributes genes that suppress rather than induce this phenotype (56). The IL-14α-transgenic mice have features not ascribed to the sle2 locus, such as increase in serum IgG and IgG2a, mesangial expansion in the glomeruli with deposition of immunoglobulins, infiltration of the salivary glands with lymphocytes, and the development of lymphoma. We observed lymphomas in the majority of IL-14α-transgenic mice by 18 mo of age. This is consistent with the fact that patients with SLE and Sjögren’s syndrome also tend to develop lymphomas late in life (37, 50, 57–59). Further studies will be necessary to determine how il14 relates to the SLE susceptibility genes that have been mapped in (NZB × NZW)F1, various NZM strains, and other SLE models.

The il14 gene is on chromosome 4 as are the majority of type 1 IFN genes. Previous studies demonstrated that IL-14 lacked antiviral effects of IFN-α (31). In the present studies we demonstrated that IL-14α-induced IgG2a, an IFN-inducible gene (60–62). IL-14α induced the production of IFN-α (Fig. 4C), but not IFN-γ, that may have been responsible for this change (60). At the same time, however, IL-12, which have recently been implicated in inducing the switch to IgG2a (63). Whether IL-14 directly induces the switch to IgG2a or expands IgG2a-producing cells will have to be determined in future studies. Type 1 IFNs have been implicated in the induction of autoimmunity in (NZB × NZW)F1 mice as well as in patients with SLE and Sjögren’s syndrome (64–66). Features of SLE are severely abrogated in (NZB × NZW)F1 mice lacking the type 1 IFN receptor (67). The generation of IL-14α-transgenic mice lacking the type 1 IFN receptor will be necessary to determine whether the activities of IL-14α require the induction of IFN-α.

The observation that IL-14α-transgenic mice developed elevated serum levels of IgM is consistent with the observation that they contain increased numbers of B1 cells and marginal zone B cells. The majority of IgM is derived from B1 B lymphocytes and marginal zone B lymphocytes, especially in mice (68–70). Previous data derived in vitro with native high molecular weight B cell growth factor/IL-14 suggested that IL-14 might act selectively on sIgDlow tonsilar B lymphocytes, which includes B1 lymphocytes and memory B2 lymphocytes (33). Our current data also suggest a role for IL-14 in IgM Ab responses to T-independent Ags, which come from B1 and marginal zone B lymphocytes.

### Table I. Comparison of B cell subpopulations in IL-14α-transgenic mice and littermate controls

<table>
<thead>
<tr>
<th></th>
<th>Peritoneal B1 Cell</th>
<th>Splenic B2 Cell</th>
<th>Marginal Zone B Cell</th>
<th>Germinal Center B Cell</th>
<th>Plasma Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littermate controls at 12 mo</td>
<td>4.9 ± 0.6</td>
<td>18.42 ± 1.6</td>
<td>1.76 ± 0.49</td>
<td>5.30 ± 1.15</td>
<td>0.48 ± 0.1</td>
</tr>
<tr>
<td>IL-14α-transgenic at 12 mo</td>
<td>14.91 ± 3.0</td>
<td>33.69 ± 4.27</td>
<td>11.78 ± 3.35</td>
<td>9.51 ± 1.65</td>
<td>1.45 ± 0.43</td>
</tr>
</tbody>
</table>

*Flow cytometry was performed on six mice as described in Materials and Methods. Peritoneal B1 cells were CD5+CD19+, IgM+, sIgDlow splenic B cells were CD19+, marginal zone B cells CD19+, CD21+, IgM+, germinal center B cells CD19+, CD38+, sIgDlow, and plasma cells CD19−, CD138+.*

FIGURE 6. A. Kidneys were harvested from IL-14α-transgenic mice and littermate controls mice at 12 mo of age. Staining with Giemsa and IgM Ab was performed as described in Materials and Methods and visualization by standard microscopy and fluorescence microscopy. Increase in mesangial cells was observed in transgenic mice but not in littermate control. Deposition of IgM in the glomeruli and blood vessel was also observed in transgenic but not in littermate control. Photos were taken at ×400. B, Parotid glands were harvested from IL-14α-transgenic mice at 8, 12, and 17 mo of age. Parotid glands were harvested from littermate control mice at 17 mo of age. Tissues were sectioned, stained with H&E, and visualized by standard microscopy, as outlined in Materials and Methods. Photographs were taken at ×400.
A Micrograph is shown of the tumors in the gastrointestinal tract of an IL-14α-transgenic mouse autopsied at the age of 17 mo. B, Staining of the tumor from an IL-14α-transgenic mouse is shown with H&E, anti-B220, and anti-CD5. C, Flow cytometry evaluation of a tumor from an IL-14α-transgenic mouse is shown with anti-CD5, anti-CD19, and anti-IgM. D, Evaluation of Ig gene rearrangements in the tumor from an IL-14α-transgenic mouse (tumor) compared with normal spleen (control) by PCR, as outlined in Materials and Methods. Arrow, The unique band in the tumor suggesting a monoclonal tumor.
The IL-14α-transgenic mice also spontaneously developed a significant increase in serum IgG and IgG2a. The IL-14α-transgenic mice have increased numbers of splenic B2 cells and germinal center B lymphocytes compared with littermate controls that would contribute to this response. They demonstrate an increased IgG response to a T-dependent Ag. The production of IgG Ab involves additional cell interactions not required for the production of IgM. The switch from IgM to IgG production requires stimulation of CD40 on B lymphocytes by CD154 on T lymphocytes or less commonly B lymphocytes, NK cells, or mast cells (71–77). IFN-γ was first described to direct H chain class switching to IgG2a (62, 78), however, the same can be accomplished with type1 IFNs (60). IL-14α may be working directly in the induction of the IgG/IgG2a response or through the stimulation of IFN-α production. IL-14α is expressed in follicular dendritic cells and this suggests that it may be somehow involved with the generation or selection of memory B lymphocytes (33). Further work will be needed to examine this issue.

The development of deposits of IgM in the kidneys and the infiltration of the parotid glands with lymphocytes in IL-14-transgenic mice suggests an SLE-like phenotype. Autoimmunity in diseases such as SLE and Sjögren’s syndrome involves excessive activation of B lymphocytes and the production of autoantibodies (79). The causes of this abnormal activation have not been fully elucidated and are likely to vary in different patients and in different animal models of these diseases. Animal models have established that alterations of individual genes that regulate lymphocyte proliferation (8, 9), inhibit apoptosis (10, 11), regulate signaling through the BCR (12–14), determine degradation or clearance of autoantigens (15, 80) or clearance of immune complexes (6, 7) can result in serum autoantibodies and immune complex-mediated glomerulonephritis. In all cases, the genetic background upon which the defined gene is added determines the severity of the disease phenotype (7, 24). The IL-14α-transgenic mice are on a B6 background that is permissive to the expression of autoimmune features. A minority of the genetically altered mice that develop a SLE-like disease also develop Sjögren’s syndrome. Sjögren’s syndrome is seen in NZW, (NZB × NZW)F1, MRL/lpr, C3H/lpr, NFS/sld, aromatase-deficient mice and I/Jic mice (81–85). All of these mice, like patients with Sjögren’s disease, develop elevated serum IgM levels (81, 84–86). The exact mechanism(s) by which IL-14 induces hypergastrinaglobulinemia is unclear. It is also unclear which autoantibodies produced in IL-14α-transgenic mice are responsible for deposition of Igs in the kidney, as high titers of anti-DNA Abs, anti-chromatin Abs, and anti-histone Abs were produced in only a minority of mice on a B6 background. Glomerulonephritis in the absence of these autoantibodies has also been observed in female NZM.C57Lc4 mice (87). Furthermore, a minority of IL-14α-transgenic mice develop Abs to Ro and La, autoantibodies that have been associated with the development of Sjögren’s syndrome (88–90). Murine models have suggested several other autoantigens may be important for the development Sjögren’s syndrome (84, 85, 91). Which autoantigens are relevant to Sjögren’s disease in IL-14α-transgenic mice is an area of current investigation.

The development of malignancies is felt to involve the dysregulation of genes involved with cell cycle regulation, growth, and/or apoptosis (92, 93). Pre-B cell lymphomas have been observed in mice overexpressing myc, especially in association with ras, raf, or bcl-1/icyclin D1 (94–96). Transgenic mice overexpressing the t(14;18) translocation including the bcl-2 gene develop large B cell lymphomas often involving dysregulated myc expression (97). The only cytokine whose overexpression alone has resulted in a B cell malignancy is IL-6, which produces plasmacytomas in transgenic mice (98). The development of lymphoma in aged IL-14α-transgenic mice was not surprising for several reasons. First, IL-14 was originally identified in a Burkitt lymphoma cell line and subsequently shown to be constitutively expressed in high-grade germinal center-derived human B cell lymphomas (31, 99). Second, IL-14 induces B cell growth and proliferation. Genes regulating B lymphocyte growth and survival are often identified to be dysregulated in B cell lymphomas (100–102). Finally, patients in their 60s and 70s with autoimmune diseases such as SLE and Sjögren’s disease often develop B cell lymphomas (48, 103, 104). The lymphomas seen in the aged IL-14α-transgenic mice most closely resembled large B cell lymphomas, which are generally derived from germinal cell B lymphocytes. It is surprising that more malignancies have not been described in the various other animal models for SLE and Sjögren’s syndrome (39, 48, 52, 57, 105).

Transgenic mice were chosen to evaluate physiological roles of IL-14α for several reasons. IL-14α is very unstable in vitro and many in vitro assays are likely affected by this property. The in vivo system allows the determination of the influence of IL-14α on intact immune systems, rather than isolated cell populations in vitro. The data resulting from these transgenic mice must, however, be viewed with several caveats. The levels of IL-14α achieved in the transgenic mice are much higher than would be seen under normal physiological conditions (Fig. 3C). The expression of IL-14α in the B lymphocyte compartment in the transgenic mice may mimic pathological more than normal physiological conditions (99, 106).

In summary, IL-14α is a highly conserved protein across species, suggesting its fundamental importance to mammalian physiology. The fact that it enhances Ab responses to vaccinations, induces autoimmunity, and contributes to the formation of B cell lymphomas suggests that it may have fundamental roles in these processes. Further work will be necessary to elucidate the mechanisms by which IL-14 participates in these disorders and to establish its roles during normal physiology.

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Disclosures

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