Rai Acts as a Negative Regulator of Autoimmunity by Inhibiting Antigen Receptor Signaling and Lymphocyte Activation

Maria Teresa Savino, Barbara Ortensi, Micol Ferro, Cristina Ulivieri, Daniela Fanigliulo, Eugenio Paccagnini, Stefano Lazzi, Daniela Osti, Giuliana Pelicci and Cosima T. Baldari

*average

Why The JI?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

References

This article cites 32 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/182/1/301.full#ref-list-1

Subscription

Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2009 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

http://www.jimmunol.org/
Rai Acts as a Negative Regulator of Autoimmunity by Inhibiting Antigen Receptor Signaling and Lymphocyte Activation

Maria Teresa Savino,* Barbara Ortensi, † Micol Ferro,* Cristina Ulivieri,* Daniela Fanigliulo,*‡ Eugenio Paccagnini,* Stefano Lazzi,§ Daniela Osti, † Giuliana Pelicci, ‡ and Cosima T. Baldari*‡

Rai (ShcC) belongs to the family of Shc adaptor proteins and is expressed in neuronal cells, where it acts as a survival factor activating the PI3K/Akt survival pathway. In vivo, Rai protects the brain from ischemic damage. In this study, we show that Rai is expressed in T and B lymphocytes. Based on the finding that Rai−/− mice consistently develop splenomegaly, the role of Rai in lymphocyte homeostasis and proliferation was addressed. Surprisingly, as opposed to neurons, Rai was found to impair lymphocyte survival. Furthermore, Rai deficiency results in a reduction in the frequency of peripheral T cells with a concomitant increase in the frequency of B cells. Rai−/− lymphocytes display enhanced proliferative responses to Ag receptor engagement in vitro, which correlates with enhanced signaling by the TCR and BCR, and more robust responses to allergen sensitization in vivo. A high proportion of Rai−/− mice develop a lupus-like autoimmune syndrome characterized by splenomegaly, spontaneous peripheral T and B cell activation, autoantibody production, and deposition of immune complexes in the kidney glomeruli, resulting in autoimmune glomerulonephritis. The data identify Rai as a negative regulator of lymphocyte survival and activation and show that loss of this protein results in breaking of immunological tolerance and development of systemic autoimmunity. The Journal of Immunology, 2009, 182: 301–308.

The Shc protein family includes four members, ShcA, ShcB/Sil, ShcC/Rai, and ShcD/RaLP, sharing a highly conserved domain organization characterized by the (CH2)-PTB-CH1-SH2 modularity (1–3). Despite their structural similarity, Shc proteins differ in tissue specificity, and genetic and biological evidence indicates that they subserve highly specific functions in the control of key cellular processes, including cell proliferation, survival, apoptosis, migration, and anoikis (1, 4–6).

Rai is expressed in the nervous system as two isoforms of 64 and 52 kDa (7, 8). ShcA and Rai display a temporal and spatial swap of gene expression during embryonic development of the CNS. ShcA is indeed highly expressed in the developing CNS; however, its levels sharply decrease in postmitotic neurons. Conversely, Rai is poorly expressed in the developing CNS but is strongly up-regulated perinatally and highly expressed in the adult brain (9). The switch of expression from ShcA to Rai is critical for neuronal development, allowing progenitor cells to proliferate in presence of ShcA and to survive and differentiate in presence of Rai (10).

Rai is phosphorylated on tyrosine residues in response to the receptor tyrosine kinase Ret and acts as an adaptor coupling the activated receptor to the PI3K/Akt signaling pathway, thereby exerting a prosurvival function in neuronal cells (4). Moreover, Rai transduces survival signals in neurons exposed to toxic insults such as serum deprivation, oxidative stress, and hypoxia (4). A study on a mouse model of ischemia/reperfusion injury has demonstrated that Rai expression protects against brain damage through activation of the PI3K/Akt pathway and inhibition of apoptosis (11). Hence Rai acts in the CNS to promote differentiation of neuronal cells and improve their survival.

In this study, we show that Rai is expressed in both T and B lymphocytes and investigate the role of Rai in lymphocyte activation and homeostasis. We show that, as opposed to neurons, Rai impairs lymphocyte survival and attenuates TCR and BCR signaling and lymphocyte activation in vitro and in vivo. Furthermore, we show that Rai−/− mice develop a systemic lupus-like autoimmune syndrome, supporting the notion that, through its inhibitory activity on Ag receptor signaling, Rai acts as a negative regulator of autoimmunity.

Materials and Methods

Mice

Rai−/− mice (9) were backcrossed with C57BL/6J mice for 12 generations, resulting in 99.8% purity (12). C57BL/6J were used as controls. The work was conducted on the Rai−/− and C57BL/6 mouse colonies bred in the animal facility at the University of Siena, where only specific pathogen free animals are admitted and periodically monitored to exclude the presence of common bacterial and viral pathogens in accordance to Federation of European Laboratory Animal Science Associations’ recommendations. With the exception of the kidney histology and proteinuria analyses, conducted on 8- to 12-mo-old mice, analyses were performed on 3- to 10-mo-old mice. Wild-type and Rai−/− mice used in each experiment were age- and sex-matched. All animal experiments were conducted in
FIGURE 1. Rai is expressed in T and B lymphocytes. A, left, Immunoblot analysis with anti-Rai mAb of spleen (SPL), thymus (Thy), and lymph nodes (LN) from control (+/+), or Rai−/− (−/−) mice. Right, Immunoblot analysis with anti-Rai mAb of postnuclear supernatants from purified splenic T and B cells. Control blots of the same filters are shown below. Representative experiments are shown (n ≥ 3). B, Flow cytometric analysis of annexin V staining of splenocytes from control (+/+), or Rai−/− (−/−) mice cultured ex vivo for 2, 4, 6, and 8 h in serum-free medium. The analysis was conducted on gated CD22+ or CD3+ cells. The graphs show the percentage ± SD of annexin V+ splenocytes (n ≥ 3; ***, p ≤ 0.001).

FIGURE 2. Splenomegaly and white pulp expansion in Rai−/− mice. A, Representative image of spleen from control (+/+), or Rai−/− (−/−) mice (left) and histograms showing weight (middle) and absolute cell numbers (right) (mean values ± SD) of spleens from control (+/+), or Rai−/− (−/−) mice (n ≥ 3; ***, p ≤ 0.001). B, Giemsa (×100) (top) and H&E (×100) (bottom) staining of spleen sections from 10-mo-old control (+/+), or Rai−/− (−/−) mice showing normal architecture in wild-type mice vs white pulp hyperplasia with florid germinal centers in Rai−/− (−/−) mice (n ≥ 3). Percentage of germinal centers in total number of follicles counted in full spleen sections (mean ± SD): 33.9% ± 9.8 in Rai−/− mice vs 15.8% ± 6.6 in control mice (n ≥ 3).

Flow cytometry
Single-cell suspensions were prepared from thymus, lymph nodes, and spleen using cell strainer filters (BD Biosciences). Peripheral blood was obtained by tail bleeding and subjected to flow cytometric analysis after hypotonic lysis of erythrocytes.

Expression of surface markers was analyzed by flow cytometry using fluorochrome-labeled mAbs (BD Biosciences; eBioscience). For the analysis of surface activation markers, cells were resuspended at 2 × 10^6/ml in RPMI 7.5% FCS and activated for 24 h either using saturating amounts of anti-CD3 mAb (2c11) purified from hybridoma supernatant and plating on plastic-immobilized secondary Abs as described (13), or by adding 30 μg/ml goat F(ab')2 to mouse IgM (Cappel; MP Biomedicals).

To measure splenic T and B cell proliferation, cells were resuspended at 20 × 10^6/ml in PBS and stained with 10 μM CFSE (Molecular Probes) for 8 min at room temperature. Cells were washed twice, resuspended at 5 × 10^6/ml in RPMI 7.5% FCS, and activated using either anti-CD3 mAb or anti-mouse IgM F(ab')2, as described above. Heat-killed Staphylococcus aureus Cowan 1 (Pansorbin; Merck) was used at 7.5–15 μg/ml. Cells were analyzed by flow cytometry 48, 72, and 96 h after stimulation, gating on CD3+ or CD22+ cells.

Apoptosis was measured by flow cytometric analysis of FITC-labeled annexin V (BD Biosciences) stained cells. Cells were resuspended at 2 × 10^6/ml in serum-free medium and either incubated as such or activated with anti-CD3 mAb or F(ab')2 to mouse IgM. Cells were analyzed after 24, 48, and 72 h, gating on CD3+ or CD22+ cells.

Flow cytometry was conducted using a FACSscan flow cytometer (BD Biosciences). Data were analyzed and plotted using FlowJo (Tree Star).

Serum processing and ELISA
Serum Abs specific for dsDNA were quantified by ELISA using a semi-quantitative kit (Alpha-Diagnostic). Serum Ig levels were determined by

agreeement with the Guiding Principles for Research Involving Animals and Human Beings and approved by the local ethical committees.

Purification of splenocytes and thymocytes, immunoprecipitation, and immunoblotting
Mice were sacrificed by cervical dislocation. Splenocytes were purified by immunomagnetic sorting using anti-panB and anti-panT Ab-conjugated beads (Dynal Biotech) and checked for purity by flow cytometry with fluorochrome-conjugated anti-CD3 and anti-CD22 mAbs. The purity was consistently >90%. Naive cells were negatively purified by immunomagnetic sorting of splenic T cells (purified with the Dynal mouse T cell negative isolation kit) or B cells (purified with the mouse B cell enrichment kit from Stem Cell Technologies) using rat anti-CD69 mAb (R&D Systems) and magnetic beads conjugated with anti-rat Ig (Invitrogen).

Cells (5 × 10^6/sample) were lysed in 1% Triton X-100 in 20 mM Tris-HCl (pH 8) and 150 mM NaCl (in the presence of a protease inhibitor mixture). Immunoprecipitation was conducted using rabbit polyclonal anti-Rai Abs (generated by the Ab Facility, Italian Foundation for Cancer Research) and protein A-Sepharose (Amersham Pharmacia Biotech). Immunoblot analysis of postnuclear supernatants was conducted by chemiluminescence (SuperSignal West Pico Chemiluminescent Substrate kit from Pierce) using as primary Abs either anti-Rai CH1 mAb (BD Biosciences) or phosphospecific Abs recognizing the active forms of ZAP70, Syk, Erk1/2, or Akt (Cell Signaling Technology), and secondary peroxidase-labeled Abs (Amersham Pharmacia Biotech). Control Abs were purchased from Santa Cruz Biotechnology. Immuneblots were scanned using a laser densitometer (Duoscan T2500 Agfa) and quantified using the ImageQuant 5.0 software (Molecular Dynamics).
ELISA using a clonotyping system-AP (Southern Biotechnology Associates) and a mouse Ig panel (Southern Biotechnology Associates). Absorbance was measured using a microplate reader (model 680; Bio-Rad) at 450 nm.

**Delayed-type hypersensitivity reaction (DTH)**

Mice were sensitized with a 3% 2,4,6-trinitrochlorobenzene (TNCB) (Sigma-Aldrich) solution (4:1 acetone:olive oil) applied to the clipped abdomen. Six days later, mice were challenged by applying 10 μl of 1% TNCB solution (9:1 acetone:olive oil) to both sides of one ear. Ear swelling was assessed 24 h later using a micrometer (ALPA) and was expressed as thickness of challenged ear minus thickness of unchallenged ear ± SD (14).

**Tissue processing and histology**

For H&E, Giemsa or periodic acid-Schiff staining tissue fragments (spleen or kidney) were fixed in 10% formalin for 24 h, embedded in paraffin, sectioned (5 μm), and stained using standard techniques. Tissues were visualized on a Zeiss microscope (Axiovert 200) equipped with a digital camera (InfinityX; Lumenera).

For immunofluorescence, kidney fragments were embedded in TissueTek OCT compound, flash-frozen in liquid N₂, and sectioned with a cryostat. Sections were stained with FITC-conjugated goat anti-mouse Ig (DAKO) and visualized by fluorescence microscopy on a Leica DMRB microscope equipped with a standard camera (using Kodak T-MAX 400 film) or a digital camera (AxioCam MRc5; Zeiss). Images were processed using the digital imaging software AxioVision (Zeiss).

**Proteinuria analysis**

The presence of proteinuria was measured in a semiquantitative way using Combur 10 test (Roche Diagnostic). Mice were scored as positive for proteinuria when protein levels exceeded 100 mg/dl.

**Results**

**Rai is expressed in T and B cells**

RT-PCR analysis identified a Rai-specific transcript in thymus and spleen of C57BL/6 and 129 mice (confirmed by automatic sequencing; data not shown). Immunoblot analysis of thymus, spleen, and lymph nodes showed that Rai is expressed both in central and peripheral lymphoid organs (Fig. 1 A). The specificity of the 52 kDa immunoreactive band, which was detectable in wild-type but not in Rai⁻/⁻ cells, was further confirmed by probing Rai-specific immunoprecipitates from splenocyte lysates with anti-Rai Abs (data not shown). Analysis of immunomagnetically sorted splenocytes showed that Rai is expressed in both T and B cells (Fig. 1 A). Surprisingly, splenic T and B lymphocytes from Rai⁻/⁻ mice were found to survive longer than their wild-type counterparts when cultured ex vivo, as assessed by annexin V staining (Fig. 1 B), indicating that, as opposed to neurons, Rai does not subserve a prosurvival function in lymphocytes.

**Splenomegaly and spontaneous T and B cell activation in Rai⁻/⁻ mice**

Beginning from ~3 mo, Rai⁻/⁻ mice developed with high frequency (>90%) splenomegaly, which was associated with increased cellularity (Fig. 2 A). Histological examination of the enlarged spleens from Rai⁻/⁻ mice revealed white pulp hyperplasia, characterized by the presence of frequent florid germinal centers.

**Statistical analyses**

Values of specified groups were compared by the Student's *t* test statistics, unpaired. A level of *p* < 0.05 was considered statistically significant.
(Fig. 2B). Flow cytometric analysis of Rai−/− splenocytes revealed a decrease in the percentage of T cells, which affected both CD4+ and CD8+ cells (Fig. 3). Conversely, an increase in the proportion of B cells was found, which affected principally mature (IgMlowIgDhigh) B cells (Fig. 3B). Males and females exhibited similar features. Absolute T cell numbers were comparable to controls in Rai−/− mice, as assessed by expression of surface activation markers. Left and middle. Representative dot plots of CD3/CD69 and CD22/CD69 labeling. Right, histograms displaying the percentage (mean value ± SD) of activated B cells (CD22+/CD69+) and T cells (CD3+/CD69+) (n ≥ 5; *** p ≤ 0.001).

Rai deficiency results in lymphocyte hyperreactivity to Ag receptor engagement

To understand whether the spontaneous activation of Rai−/− lymphocytes may reflect an inhibitory function of Rai on Ag receptor signaling, the impact of Rai expression on this process was assessed. Rai−/− T cells responded more vigorously to TCR engagement compared with T cells from control mice, as assessed by measuring ZAP-70, Erk1/2 phosphorylation (Fig. 5A) and Ca2+ mobilization (data not shown). Of note, Erk activation was comparable in control and Rai−/− T cells stimulated with PMA (data not shown), indicating that Rai specifically interferes with TCR coupling to the MAPK cascade. Similarly, BCR signaling was enhanced in Rai−/− B cells, as shown by the higher levels of Syk and Erk1/2 phosphorylation (Fig. 5B), as well as enhanced Ca2+ mobilization (data not shown) triggered by surface IgM cross-linking. Akt phosphorylation in response to TCR or BCR engagement was also enhanced in Rai−/− splenocytes compared with controls (Fig. 5C). This was paralleled by increased resistance of activated T and B cells to apoptosis (Fig. 5D). Enhanced TCR and BCR signaling was also observed, albeit to a lower extent, in purified naive Rai−/− splenocytes, as assessed using as a read-out Erk phosphorylation (data not shown), ruling out the possibility that the enhanced response of Rai−/− T and B cells results solely from the higher proportion of spontaneously activated lymphocytes in these mice.

FIGURE 4. Spontaneous peripheral lymphocyte activation in Rai−/− mice. Flow cytometric analysis of lymphocytes from spleen (SPL) (A), lymph nodes (LN) (B), and peripheral blood (PBMC) (C) from age-matched control (+/+) or Rai−/− (−/−) mice stained with fluorochrome-labeled Abs specific for the indicated cell surface markers. Left and middle. Representative dot plots of CD3/CD69 and CD22/CD69 labeling. Right, histograms displaying the percentage (mean value ± SD) of activated B cells (CD22+/CD69+) and T cells (CD3+/CD69+) (n ≥ 5; *** p ≤ 0.001).
cells proliferating in response to anti-IgM Abs, alone or in combination with *S. aureus* Cowan 1, was consistently higher than control B cells at all time points analyzed (Fig. 6B). T cell proliferation was also significantly increased compared with controls when splenocytes from Rai⁻/⁻ mice were stimulated with anti-CD3 mAb (Fig. 6C).

The impact of Rai expression on the response to Ag in vivo was assessed in a DTH assay, which measures hapten-specific T cell responses. A highly significant increase in the DTH response was observed in Rai⁻/⁻ mice (Fig. 6D). Taken together with the enhanced spontaneous and Ag receptor-dependent responses of Rai⁻/⁻ T and B cells in vitro, this finding supports the notion that Rai acts as a negative regulator of lymphocyte activation.

**Development of autoimmune glomerulopathy in Rai⁻/⁻ mice**

Spontaneous activation of autoreactive peripheral lymphocytes is a feature associated with autoimmunity. To assess the existence of activated self-reactive cells in Rai⁻/⁻ mice, sera were tested by ELISA for the presence of autoantibodies. Anti-dsDNA Abs were found in a significant proportion of Rai⁻/⁻ mice (~80% vs ~8% in controls) (Fig. 7A) and correlated with the presence of splenomegaly. The proportion of peritoneal B-1 cells, which are believed to be the source of low-affinity polyclonal autoantibodies (15), was also increased in Rai⁻/⁻ mice (Fig. 7B). Furthermore, analysis of serum immunoglobulins revealed an increase in the concentrations both of IgM and of IgG, which involved principally the IgG2a and IgG3 isotypes (Fig. 7C).

Circulating anti-dsDNA Abs are a hallmark of lupus-like autoimmune syndromes. These autoantibodies deposit as immune complexes in kidney glomeruli, resulting in tissue damage and development of glomerulonephritis (16, 17). Immunofluorescence analysis of kidney sections from Rai⁻/⁻ and control mice using fluorochrome-labeled anti-mouse IgG Abs revealed intense granular staining in glomeruli of Rai⁻/⁻ mice, consistent with deposition of immune complexes (Fig. 7D). Histological analysis of kidneys from 8- to 12-mo-old Rai⁻/⁻ mice showed that, while the tubular architecture was normal, glomeruli displayed a dramatic alteration in appearance characterized by the presence of thick crescent-like structures of amorphous proteinaceous material, which is some instances included cellular components, deposited in Bowman’s space, with resulting collapse of the glomerular tuft (Fig. 7E) (data not shown).

To address the impact of the glomerular alterations on renal function, the urine from 8- to 12-mo-old Rai⁻/⁻ mice was analyzed for the presence of proteins. As opposed to wild-type controls, which did not develop proteinuria (n = 0/24), proteinuria (>100 mg/dL) was observed in >20% of Rai⁻/⁻ mice (8/35 Rai⁻/⁻ mice, of which 2 mice were 8 mo old, 3 mice were 10 mo old, and 3 mice were 12 mo old), indicating that the glomerular damage results in compromised renal function in these mice. The levels of proteinuria were highest in mice with the most marked glomerular alterations and splenomegaly (data not shown).

**Discussion**

Our findings provide two important new pieces of information on what has been to date considered a brain-specific adaptor, but which recent reports have identified in other tissues including enteric glial cells, endothelial cells, and smooth muscle cells of the gastrointestinal tract (18). First, Rai mRNA and protein can be detected in both T and B lymphocytes. Although expression is
lower than in neuronal cells, the hyperreactivity of Rai−/− lymphocytes both in vivo and in vitro demonstrates that the low amounts of constitutively expressed Rai are sufficient to elicit a biological output. A similar reassessment of tissue specificity has been reported for the related protein, p66ShcA, which was believed to be absent in hematopoietic cells but has been shown to be expressed in T cells, B cells, and monocytes (13, 19). Moreover, expression of the adaptor LAB, initially identified as the functional homologue of LAT in B cells, has been demonstrated also in activated T cells (20). Second, Rai appears to subserve different functions in the control of cell survival in lymphocytes and neurons. Rai expression correlates indeed both with enhanced spontaneous apoptosis and with enhanced activated cell autonomous death, a form of apoptosis executed by the mitochondrial pathway which activated lymphocytes undergo in the absence of survival signals (21). A similar striking instance of tissue specific function has been described for LAB, which acts as a positive regulator of BCR signaling and as a negative regulator of TCR signaling (20, 22).

The role of PI3K signaling in T and B cell activation and survival is as yet debated. Pharmacological and genetic dissection of PI3K in lymphocytes has indeed resulted in conflicting results as to the function of PI3K as a positive or negative regulator of Ag receptor signaling (23). The enhancement in Akt phosphorylation in Rai−/− lymphocytes supports the notion that PI3K is implicated in the positive control of Ag receptor signaling and is consistent with the functional link between Rai and PI3K observed in neuronal cells (4). However, while in neuronal cells Rai couples the Ret tyrosine kinase receptor to PI3K activation through interaction with the p85 regulatory subunit of the enzyme (4), in lymphocytes Rai appears to attenuate TCR- and BCR-dependent PI3K activation. Interestingly, T cells lacking the adaptor LAX, which interacts with p85, also display enhanced Akt phosphorylation (24). The authors suggest the possibility that LAX could compete for binding of p85 to other proteins implicated in PI3K activation. A similar mechanism may account for the enhancement in TCR signaling observed in LAX−/− B cells (23). The authors suggest the possibility that LAX could compete for binding of p85 to other proteins implicated in PI3K activation. A similar mechanism may account for the enhancement in TCR signaling observed in LAX−/− B cells (23).
by inhibiting initiation of Ag receptor signaling (as indicated by the enhanced ZAP-70 and Syk phosphorylation in Rai-deficient lymphocytes), Rai is expected to uncouple the TCR and BCR from multiple signaling pathways, including the PI3K/Akt pathway. In this scenario, the adaptor function of Rai in PI3K recruitment to the activated receptor identified in neuronal cells would not be functionally relevant in lymphocytes and the link with PI3K activation indirect.

Potential clues to the mechanisms underlying the antimitogenic activities of Rai in lymphocytes may be provided by the related adaptor p66ShcA. We have reported that p66ShcA blunts Ras/MAPK activation by competitively inhibiting p52ShcA recruitment to the activated TCR (13). This involves the phosphoryrosine binding domains of p66ShcA, which are conserved among Shc family members. Since the affinity of Rai for Grb2/Sos is significantly lower compared with p52ShcA (25), Rai may be hypothesized to act as a dominant inhibitor of Ras activation by p52ShcA. This possibility could be consistent with the enhanced Ag receptor-dependent Erk activation in Rai<sup>−/−</sup> lymphocytes. In contrast, the observation that Rai deficiency results in increased ZAP-70 and Syk phosphorylation in response to Ag receptor engagement suggests that Rai may prevent their activation by interfering with their binding to the phosphorylated ITAMs in the activated receptors. Results obtained in Rai overexpressing Jurkat cells, showing Rai association with the activated TCR and decreased CD3ζ/ZAP-70 association, support this possibility (M. Ferro et al., manuscript in preparation).

Lymphocyte development, selection, and homeostasis are critically controlled by a fine balance of signals regulating cell proliferation and survival. The spontaneous onset of autoimmunity in Rai<sup>−/−</sup> mice suggests that Rai might play an important function in the control of this balance. Loss of the inhibitory activity of Rai on TCR and BCR signaling in Rai<sup>−/−</sup> mice may facilitate the activation, proliferation, and survival of the rare peripheral self-reactive lymphocytes that have escaped negative selection in the thymus, leading to accumulation of activated self-reactive lymphocytes in the periphery and eventual development of autoimmunity. In this context, the limited effect of Rai deficiency on the number of splenic T cells in Rai<sup>−/−</sup> mice, despite their state of enhanced spontaneous activation, is intriguing. The following scenarios could be envisaged: i) because of the hyperreactivity of the TCR, endogenous peptide ligands might be able to promote activation but not cell cycle progression of weakly self-reactive T cells in the absence of additional stimuli and ii) Rai may interfere with only a subset of the pathways normally triggered by the TCR in vivo. Release of these pathways in Rai-deficient T cells could lead to an alteration in the balance of signals evoked by the TCR, resulting in an incomplete biological output. In contrast, the large expansion of B cells, which is characteristic of the germinal center reaction, is likely to be further enhanced in Rai<sup>−/−</sup> mice by the combined contribution of enhanced T cell help and enhanced BCR signaling. Of note, we have recently reported that lymphocytes from mice deficient of the related adaptor, p66ShcA, also display hyperreactivity to Ag receptor agonists, which results in enhanced proliferative responses and spontaneous development of autoimmunity (19). Rai and p66Shc appear, therefore, to participate both in setting the threshold for TCR and BCR signaling.

The autoimmune phenotype in Rai<sup>−/−</sup> mice includes the following pathological features: i) lymphoid hyperplasia, characterized by active germinal centers in the spleen and accumulation of spontaneously activated lymphocytes which survive longer when cultured ex vivo, ii) increased IgM and IgG, with a preferential increase in the IgG2a and IgG3 isotypes, which have been implicated in glomerular damage for their capacity to efficiently activate complement and interact with FcyRI and FcγRIII (26), iii) production of Abs against dsDNA, the principal self Ag in systemic lupus erythematosus, and iv) glomerular deposition of immune complexes, associated with formation of matrix crescents and development of glomerulonephritis. These features are consistent with a lupus-like autoimmune disease. Of note, similar pathological findings were observed in the 129sv genetic background (unpublished results). The pathology spontaneously developed by Rai<sup>−/−</sup> mice is significantly less severe than in the classical models of murine lupus, the New Zealand Black/ New Zealand White mouse (27) and the Fas-deficient MRL/lpr mouse (28). A possible explanation is that, as opposed to other genetic backgrounds including the mixed C57BL/6 × 129sv background where low levels of antinuclear immunity and mild immune deposits at an advanced age have been recorded, neither the C57BL/6 nor the 129sv genomes are significantly prone to autoimmunity (29). Moreover, the spontaneous autoimmune phenotype displayed by mice lacking other components of the TCR or BCR signaling machinery is, in some instances, also relatively mild (30–33), suggesting a redundancy in at least in some of the molecules implicated in the regulation of individual pathways (e.g., the Ras/MAPK pathway). Finally, based on the substantial overlap in the activities of Rai and p66ShcA both in vitro and in vivo, it could be hypothesized that Rai deficiency may be partly compensated by p66ShcA. Specific differences in the pathology developed by p66Shc<sup>−/−</sup> mice compared with Rai<sup>−/−</sup> mice (later disease onset and autoimmune alopecia in p66Shc<sup>−/−</sup> mice, membranous glomerulonephritis in p66Shc<sup>−/−</sup> mice vs crescential glomerulonephritis in Rai<sup>−/−</sup> mice) (19) suggest, however, that p66ShcA and Rai subserve at least some unique functions in lymphocytes.

In summary, the data presented in this paper highlight a novel role of Rai in the immune system, where it appears to act as a negative regulator of mitogenic and prosurvival signals emanated from Ag receptors in T and B cells. The development of systemic autoimmunity in Rai<sup>−/−</sup> mice supports the relevance of this activity of Rai to the maintenance of tolerance and to lymphocyte homeostasis.

Acknowledgments

We thank S. Grassini and L. F. Falco for technical assistance, S. Feti and the mouse facility at Italian Foundation for Cancer Research Institute of Molecular Oncology Foundation-European Institute of Oncology Campus for animal care, and M. M. D’Elios, L. Leoncini, F. Forconi, A. Cavani, M. Rescigno, A. Gobbi, and J. L. Telford for productive discussions.

Disclosures

The authors have no financial conflict of interest.

References

SPONTANEOUS AUTOIMMUNITY IN Rai−/− MICE


