E2f1 Mutation Induces Early Onset of Diabetes and Sjögren's Syndrome in Nonobese Diabetic Mice

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E2f1 Mutation Induces Early Onset of Diabetes and Sjögren’s Syndrome in Nonobese Diabetic Mice

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E2f1 is an important regulator of T cell proliferation, differentiation, and apoptosis that controls the transcription of a group of genes that are normally regulated at the G1 to S phase transition in the cell cycle. Insulin-dependent diabetes mellitus (IDDM) and Sjögren’s syndrome (SS) are highly regulated autoimmune diseases that develop spontaneously in NOD mice. The aim of the present in vivo study was to explore the functional importance of the E2f1 molecule in IDDM and SS, in the context of whole animal physiology and pathophysiology, using E2f1-deficient NOD mice. For the experiment, we produced NOD mice homozygous for a nonfunctional E2f1 allele onto a NOD background. E2f1-deficient NOD mice developed an early and increased onset of diabetes as compared with their littermates. These mice also exhibited a defect in T lymphocyte development, leading to excessive numbers of mature T cells (CD4+ and CD8+), due to a maturation stage-specific defect in the apoptosis of thymocytes and peripheral T cells. We also found that they also exhibited a more rapid and increased entry into the S phase following antigenic stimulation of spleen cells and thymocytes in vitro. Furthermore, E2f1-deficient mice showed a profound decrease of immunoregulatory CD4+CD25+ T cells, while the spleen cells of NOD mice lacking E2f1 showed a significant increase of the proinflammatory cytokine IFN-γ following antigenic stimulation in vitro. Consistent with these observations, E2f1 homozygous mutant NOD mice were highly predisposed to the development of IDDM and SS. The Journal of Immunology, 2004, 173: 4908–4918.

The Journal of Immunology

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The Journal of Immunology
(39) may inactivate the E2f1-DP1 complex. The decision by individual cells to proliferate, remain quiescent, or die is crucial to all organisms. Dysregulation of the normal functions involved with this decision can result in failure of important reparative, inflammatory, or other adaptive responses and can lead to aberrant proliferation characteristics of late-onset autoimmune diseases due to the deposition of T lymphocytes in different glands and salivary gland dysplasia by destruction of acinar cells (40–43). Several recent studies demonstrated that a mutation of the E2f1 gene in mice causes enhanced T lymphocyte proliferation, leading to testicular atrophy, splenomegaly, salivary gland dysplasia, and other systemic and organ-specific autoimmunity (40–45). However, it is uncertain whether the function of E2f1 has an influence in the development of IDDM and SS. To address this issue, we generated E2f1-deficient NOD mice. Furthermore, to test the critical function of the E2f1 molecule in the development of IDDM and SS in NOD mice in vivo, we inactivated the E2f1 locus by homologous recombination.

Materials and Methods

Generation of E2f1-deficient NOD mice

NOD/LtJ and E2f1−/− mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME). They were housed in polypropylene mouse cages with pine chips and maintained in accordance with guidelines established by the National Institute of Infectious Diseases. The E2f1−/− allele was functionally disrupted by insertion of a neomycin-resistant gene (neoR) that had been backcrossed from the original chimeric stock and mixed with the 129/SV and C57BL/6 genome (42) onto the NOD inbred background. Het had been backcrossed from the original chimeric stock and mixed with the Laboratory (Bar Harbor, ME). They were housed in polypropylene mouse

Table I. Linkage markers analyzed to fix NOD-derived Idd loci to homozygosity in NOD.E2f1−/− congenic mice

<table>
<thead>
<tr>
<th>Idd locus/Chromosome</th>
<th>Linkage Marker</th>
<th>Homozygous for NOD Allele</th>
<th>Relative</th>
<th>Microsatellite Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idd1/17</td>
<td>D17Mts28</td>
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<td></td>
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<td></td>
<td>D17Mts34</td>
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<tr>
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<td></td>
<td>D17Mts62</td>
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<td>E2 = NOD</td>
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<tr>
<td></td>
<td>D2Mts257</td>
<td>E2 &lt; NOD</td>
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<td></td>
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<tr>
<td></td>
<td>D2Mts268</td>
<td>E2 &lt; NOD</td>
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<td></td>
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<tr>
<td>Idd14/13</td>
<td>D15Mts17</td>
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<tr>
<td>Idd15/15</td>
<td>D5Mts48</td>
<td>E2 &gt; NOD</td>
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</table>

* Microsatellite markers with the indicated allelic size variants were typed in N5 backcross mice used for the intercross.

Table II. Frequency of homozygosity in N5 intercross generation mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Number</th>
<th>% of Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD.E2f1−/−+</td>
<td>33</td>
<td>24.44 (n = 135)</td>
</tr>
<tr>
<td>NOD.E2f1−/−-</td>
<td>33</td>
<td>24.44 (n = 135)</td>
</tr>
<tr>
<td>NOD.E2f1−/−n</td>
<td>69</td>
<td>51.12 (n = 135)</td>
</tr>
</tbody>
</table>

DNA samples from the N5 intercross generation were typed by genomic PCR and the results demonstrated that NOD.E2f1−/−+, NOD.E2f1−/−−, and NOD.E2f1−/−n mice were produced at a 1:1:2 ratio.
10^6/ml in 24-well plates in culture medium (RPMI 1640 supplemented with glutamax, 10% FBS, antibiotics, 10 mM HEPES, and 5 × 10^-5 M 2-ME) in the presence of soluble anti-CD3 Ab at 1 μg/ml for 4 h. During the final 45 min of culture, the cells were labeled with 10 μM BrdU. The cell cycle positions and active DNA synthetic activities of the cells were determined by analyzing the correlated expression of total DNA and incorporated BrdU levels. BrdU-incorporated cells were stained with FITC-anti-BrdU Ab and the total DNA content was stained with 7-aminoactinomycin D using a BrdU Flow kit (BD Pharmingen, San Diego, CA), after which they were analyzed with FACSScan CellQuest software (BD Biosciences, San Jose, CA).

Abs and chemicals

The following mAbs were purchased from BD Pharmingen: FITC-conjugated anti–mouse anti-CD4 (H129.19), anti–CD8a (53-6.7), anti–CD25 (g4-4E8), allophycocyanin-conjugated anti–CD4 (H129.19), anti–CD8a (53-6.7), anti–CD25 (g4-4E8), and IL-4 by CD4+ T cells after 4 h of culture in the presence of leukocyte activation kits (BD Pharmingen). Cells were analyzed using a FACScan flow cytometer and the CellQuest program. Samples were gated on the forward light scatter and side light scatter and were used to identify viable lymphocytes and their ratios.

Adoptive type 1 diabetes transfer experiments

Spleen cells of 13 NOD.E2f1+/+ and 13 NOD.E2f1−/− female mice from 5- to 10-wk-old were isolated in RPMI 1640 medium supplemented with 10% FBS. RBC were depleted by a lysis with ACK buffer (0.155 M ammonium chloride, 0.1 M disodium EDTA, and 0.01 M potassium bicarbonate, pH 7.2). After washing, counting of the total cell numbers, and viability evaluation, splenocytes (5 × 10^6) were i.p. injected into 26 eight- to 10-wk-old NOD/SCID female mice, respectively. Beginning 1–2 wk after adoptive cell transfer, the recipient animals (NOD/SCID) were monitored twice a week for clinical onset of diabetes that was determined by the presence of glucose in the urine and blood. Urine was tested with Ames reagent strips and confirmed positive by blood glucose measurements. The
recipient animals were killed at the time they became diabetic and the pancreas and salivary glands were subjected for histological analyses.

**Results**

**Establishment of the NOD.E2f1−/− mouse strain**

Heterozygous carriers of the E2f1−/− allele from the backcross generation and all progeny of the N5 intercross generation were typed by PCR for the microsatellite markers shown in Table I, which were closely linked to the indicated Idd loci. The SSLP analyses identified mice homozygous for allelic variants characteristic of NOD mice at all of the Idd1–Idd15 linkage markers (Table I). Typing of these markers confirmed the homozygous presence of the NOD-derived genome at all identified Idd loci in the N5 progenitors used for the intercross (Table I). Genotyping by genomic PCR demonstrated that E2f1 mutant-type (NOD.E2f1−/−), heterozygous (NOD.E2f1+/−), and wild-type (NOD.E2f1+/+) animals were produced at the expected ratio of 1:2:1 (Table II). Homozygous E2f1−/− mice of the fifth (N5) backcross intercross generation were used to establish the NOD.E2f1−/− mouse strain, which was maintained by brother-sister mating. To discuss the potential for the autoimmune effects to be linked in NOD mice to allelic E2F-1 polymorphism, the original mouse strains (C57BL/6 and 129sv) to the knockout mouse were compared with NOD mice by SSLP analysis. As a result, there were no differences in the E2F-1 polymorphisms between NOD and the original strains.

**Early and increased incidence of diabetes in E2f1-deficient NOD mice**

E2f1−/− mice were bred onto a NOD background and diabetes was assessed by weekly measurement of urinary glucose or blood glucose concentration. The incidence of diabetes was ~88% in female N5 mutant (NOD.E2f1−/−) (Fig. 1a) and 58% in male N5 mutant (NOD.E2f1−/−) mice (Fig. 1b), while 71% of the N5 wild-type (NOD.E2f1+/+) females and 25% of the N5 wild-type (NOD.E2f1+/+) males became diabetic by 32 wk of age (Fig. 1, p < 0.05 and p < 0.001, respectively). Disease onset was earlier and more severe in mutant NOD.E2f1−/− mice as compared with their wild-type littermates (Fig. 1). The incidence of diabetes in both heterozygote male and female N5 (NOD.E2f1−/+) mice was not significantly different as compared with the N5 wild-type (NOD.E2f1+/+) control mice (Fig. 1).

**Low salivary flow rate in E2f1-deficient NOD mice**

Salivary analyses of different mouse strains showed that the total amount of secreted saliva was significantly lower in both NOD and E2f1−/− as compared with the standard (NOD × B10.D2) F1 mice after stimulation by the secretagogue (Fig. 2). Our salivary analyses showed that the total amount of secreted saliva after stimulation by the secretagogue was significantly lower in N5 mutant (NOD.E2f1−/−) mice (380.0 ± 24.5 μg/100 g body weight) as...
FIGURE 4. Analyses of insulitis in NOD.E2f1−/− and NOD.E2f1+/+ group mice at 12–16 wk of age. The degree of mononuclear cell infiltration was graded as follows: grade 0, no infiltrating cells in the islets; grade 1, infiltrating cells adjacent to the islets but not in the islets; grade 2, infiltrating cells occupying <25–50% of the islets’ area; grade 3, infiltrating cells occupying 25–50% of the islets’ area; grade 4, infiltrating cells occupying >50% of the islets’ area. The assessment of insulitis was performed using H&E-stained pancreas sections. Results are expressed as the mean ± SD of the insulitis counts for six independent mice selected randomly. The asterisk denotes significantly different insulitis counts between two groups (p < 0.001).

Histological abnormalities of exocrine glands in E2f1-deficient NOD mice

Histological examinations of submandibular salivary gland and pancreas specimens from 12- to 16-wk-old E2f1-deficient N5 mutant prediabetic NOD mice revealed that large numbers of mononuclear inflammatory cells had infiltrated the salivary glands and pancreas islets with some evidence of acinar cell degeneration (Fig. 3, a and c). Abnormally large nuclei or nuclei doubled in number were observed in pancreas islets of N5 (NOD.E2f1−/−) mice (Fig. 3a). This morphology represents a more severe destructive cellular infiltration of the tissue and suggests that the E2f1 molecule may help to maintain a normal nuclear structure and possible DNA content within these organ tissues (40, 42). The histological examinations also revealed that severe insulitis in the mutant NOD.E2f1−/− group (grade 0, 20.9 ± 7.9%; grade 1, 14.8 ± 5.9%; grade 2, 16.1 ± 7.6%; grade 3, 20.9 ± 4.5%; and grade 4, 27.0 ± 5.7%) as compared with the wild NOD.E2f1+/+ group (grade 0, 26.3 ± 4.5%; grade 1, 28.3 ± 6.4%; grade 2, 16.9 ± 0.6%; grade 3, 10.1 ± 2.5%; and grade 4, 16.5 ± 2.2%; Fig. 4). In particular, there were larger differences in grade 3 and grade 4 insulitis between these two groups. Furthermore, we analyzed whether the splenocytes of young E2f1-deficient NOD and standard NOD mice can induce lymphocytic infiltration in salivary glands and pancreas and development of diabetes by spleen T cells of NOD.E2f1−/− mice transferred into NOD.SCID mice with differing efficiencies. As expected, the majority (75%) of recipients injected with splenocytes from both E2f1-deficient NOD and NOD mice became diabetic by 10 wk after transfer (data not shown). There was no significant difference in the onset of diabetes between the recipients.

Thymus, thymocyte, and peripheral T cell abnormalities in NOD.E2f1−/− mice

The E2f1 molecule was found to play an important role in normal thymic development. The thymi of 5-wk-old N5 mutant-type NOD.E2f1−/− mice were noticeably enlarged compared with those of N5 wild-type littermates (NOD.E2f1+/+), and thymus weight per 100 g body weight in E2f1-deficient NOD mice was also increased (26.2 ± 2.3) compared with their littermates (18.7 ± 0.9; Fig. 5a, p < 0.001). This increased thymus size reflected a consistent increase in thymic cellularity, demonstrated by an ~55% increase in the number of thymocytes per thymus (Fig. 5b, p < 0.001). Several mechanisms might explain the increased number of thymocytes. To investigate the mechanisms of thymus and thymocyte abnormalities, the developmental profiles of thymocytes from N5 (NOD.E2f1−/−) mice and N5 wild-type (NOD.E2f1+/+) mice were compared. By monitoring the expression of CD4 and CD8 cell surface markers, the extent of thymocyte maturation was assessed. As thymocytes mature, they progress sequentially through double-negative (CD4−CD8−), double-positive (CD4+CD8−), and single-positive (CD4+CD8− or CD4−CD8+) stages. Upon staining with anti-CD4 and anti-CD8 Abs, thymi of 5-wk-old N5 (NOD.E2f1−/−) mice were consistently found to contain a higher fraction of mature thymocytes (CD4+ or CD8+) than their N5 wild-type (NOD.E2f1+/+) littermates (Fig. 6, d–f, p < 0.01). Furthermore, the total number of thymocytes per thymus as well as the absolute number of cells in all thymocyte populations were increased in N5 (NOD.E2f1−/−) mice (Figs. 5b and 6). There were also significantly low numbers of double-positive (CD4+CD8+) thymocytes in N5 (NOD.E2f1−/−) mice (72.9 ± 2.9%) as compared with the wild-type NOD.E2f1+/− mice (79.0 ± 1.6%; Fig. 6, d and e, p < 0.01). Finding from flow...
cytometric analyses of spleen and lymph node T cells revealed that the percentages of mature T cells (CD4+ and CD8+) were significantly increased in N5 (NOD.E2f1−/−) mice than in wild-type N5 (NOD.E2f1+/+) mice (Fig. 6, a–c, g, h, and i, p < 0.01), and the absolute number of spleen T cells (CD4+ and CD8+) was also significantly increased in mutant NOD.E2f1−/− mice as compared with wild-type NOD.E2f1+/+ mice (Fig. 6k). These results indicated that E2f1-deficient NOD mice also have an increased number of mature peripheral T cells. Increased cell numbers in the peripheral tissues may be due to increased proliferation, decreased cell death as well as or in place of alteration of cell migration from the thymus in E2f1-deficient NOD mice.

**Increased entry into S phase of cell cycle in E2f1-deficient NOD mice**

Thymocyte and spleen cell proliferation in vitro in response to stimulation with mitogens was found to be distinguishable when cells isolated from 5-wk-old mutant (NOD.E2f1−/−) and wild-type (NOD.E2f1+/+) mice were compared (Fig. 7). Flow cytometric analyses of the cell cycle positions and active DNA synthetic activities of the cells revealed that the fractions of cells in the S phase in the thymus and spleen of N5 (NOD.E2f1−/−) mice were significantly increased (29.1 ± 3.6 and 11.5 ± 2.3, respectively) as compared with those in the wild-type N5 (NOD.E2f1+/+) mice.
Results of deficient NOD mice are more susceptible to IDDM and SS than wild-type NOD. E2f1-decient NOD mice compared with those in the wild-type N5 (NOD.E2f1+/+) mice (72.7 ± 8.6 and 91.2 ± 3.3, respectively; Fig. 7, b and d, p < 0.05 and p < 0.005, respectively). Furthermore, significant differences in cells in the G2 + M phase in the spleen were seen between the mutant-type NOD.E2f1−/− mice and wild-type NOD.E2f1+/+ mice (Fig. 7d, p < 0.05); however, there were no significant differences in the thymus between these two groups (Fig. 7b, p = 0.19).

CD4+CD25+ T cell subset reduced in number in E2f1-deficient NOD mice

Results of flow cytometric analyses of the CD4+CD25+ T cell subset revealed that a significant decrease of these cells in the spleen was observed in the 5-wk-old E2f1-deficient NOD mice compared with that in the 5-wk-old wild-type NOD.E2f1+/+ mice (Fig. 8, a and b, p < 0.01). Furthermore, the total number of the immunoregulatory CD4+CD25+ T cells in spleen were also decreased significantly in NOD.E2f1−/− (28.3 × 10⁶ cells/mouse) mice as compared with the wild-type NOD.E2f1+/+ (62.4 × 10⁶ cells/mouse) mice (p = 0.007, data not shown in figure). There was no significant difference in CD4+ CD25+ T cells in the lymph nodes and thymus between these two groups of mice, although a slightly lower number of this subset was found in the mutant NOD.E2f1−/− mice as compared with the wild-type NOD.E2f1+/+ mice (Fig. 8, c–f). These results reflect that E2f1-deficient NOD mice are more susceptible to IDDM and SS than wild-type NOD.E2f1+/+ mice.

Th1 and Th2 cytokines increased in E2f1-deficient NOD mice

The roles of cytokines in autoimmune diabetes and SS have been extensively studied in NOD mice. Some cytokines, such as IFN-γ, are pathogenic, whereas others, like IL-4, are able to block the development of autoimmune diseases such as IDDM and SS. Spleen cells from E2f1-deficient NOD mice were stimulated with anti-CD3 mAb, and the supernatants were harvested to determine the levels of IL-4 and IFN-γ production. The production of IFN-γ by spleen T cells in 5-wk-old E2f1-deficient NOD mice was significantly increased (840.0 ± 216.7) as compared with the wild-type NOD.E2f1+/+ mice (443 ± 22.8; Fig. 9, p < 0.005). Furthermore, Th2 cytokine IL-4 production by spleen T cells in the mutant NOD.E2f1−/− mice was slightly increased (117.0 ± 18.2) as compared with the wild-type NOD.E2f1+/+ mice 98.0 ± 34.0); however, the difference was not significant (Fig. 9, p = 0.38).

Discussion

To investigate the functional role of the E2f1 molecule in IDDM and SS in NOD mice, we generated an E2f1-deficient NOD mouse (NOD.E2f1−/−) strain. Several studies have demonstrated that E2f1-deficient animals develop late-onset autoimmune features and are characterized by widespread inflammatory infiltrates, exocrine gland dysplasia, glomerular immunocomplex deposition, and hyperplastic thymic and peripheral T lymphocyte proliferation (40, 41, 43, 45–47). In the present study, our analyses of SSLP by PCR using microsatellite markers and neo8 gene primers demonstrated that E2f1 homozygous mutant, heterozygous, and wild-type mice were produced at the expected ratio of 1:2:1 (Table II) (2, 41, 42).
The E2f-1 polymorphism was not found in the autoimmune strain (NOD) and nonautoimmune strains (C57BL/6 and 129sv). The SSLP analysis using the D2Mit286 marker as the Idd13 on chromosome 2 that is close to the E2F-1 gene did not show the polymorphism in NOD, E2f1/H11002/H11002, E2f1/H11002/H11002, 129sv, and C57BL/6 mice in our experiments. Our results also showed that the E2f1 mutation in NOD mice caused early and increased incidences of IDDM and SS (Fig. 1).

At 5 wk old, N5 (NOD.E2f1/H11002/H11002) mice displayed a significant enlargement of the thymus, spleen, and lymph nodes as compared with their littermates. Enlargement of these organs reflects an expansion in the population of mature CD4/CD8 single-positive thymocytes and peripheral T cells, which may have been due to a maturation stage-specific defect in the normal pathway of T cell apoptosis (40–42). Recently, apoptosis was shown to be an important event during normal thymocyte maturation (40, 41). The elimination of thymocytes bearing self-reactive T cell receptors occurs during negative selection and is believed to be mediated by TCR-stimulated apoptosis of CD4/CD8 double-positive thymocytes just before their conversion into mature thymocytes (40, 41, 48, 49). Those observations, taken together with our finding that NOD.E2f1/H11002/H11002 mice have a defect in TCR-stimulated apoptosis of CD4/CD8 double-positive thymocytes, raise the possibility that the E2f1 molecule is functionally active in the process of negative selection.

Recent studies have found that E2f1 functions primarily as a suppressor of cell proliferation (40, 41, 50). Consistent with these findings is the observation that E2f1 DNA binding sites in the promoters of several cell cycle genes function primarily to repress transcription of these genes during G0 and G1 (51–53), presumably...
by binding to the E2f1-Rb complex. Furthermore, several studies have suggested that repression of transcription the E2f1-Rb complex suppresses entry into the S phase (40, 41, 54). Thus, E2f1-deficient cells may be unable to tether Rb to E2f1 DNA binding sites during the G0 or G1 phase, which may lead to an aberrant expression of particular cell cycle regulatory genes and inappropriate cell proliferation (40). A role of E2f1 in the suppression of thymocyte cell cycle progression might explain the excessive proliferation and significantly different cell cycle distribution seen in the thymus and spleen T lymphocytes of 5-wk-old N5 (NOD.E2f1+/−/−) mice as compared with the wild-type NOD.E2f1+/−/+ mice (Fig. 8). Moreover, a direct role of E2f1 as a suppressor of cell cycle progression may explain the additional phenotypes that we and other investigators observed in E2f1-deficient mice, such as testicular atrophy, hypposalivation or exocrine gland dysplasia, increased mortality, hair loss, and sporadic skin tumor formation (40–43). These results indicate that a deletion of the E2f1 molecule leads to enhanced T cell proliferation and suggest that excessive infiltration of T lymphocytes in pancreas islets and salivary glands caused severe IDDM and SS in E2f1-deficient NOD mice as compared with the wild-type NOD.E2f1+/−/+ mice (Fig. 2). A role of apoptosis of epithelial cells in the effector phase of sialoadenitis in the NOD mouse was suggested after the study of some investigators (52–57), while other investigators did not find evidence for a role of apoptosis in the effector phase of sialoadenitis (58). In addition, in our experiments we did not find evidence for a role of apoptosis in the initiation phase of IDDM and SS in either group of mice. E2f1 deficiency may regulate proliferative activities of T cells and associate in the initiation phase rather than promotion of apoptosis. Therefore, the induction of autoreactive T cells was proposed as an important mechanism in the initiation phase of the disease in NOD mice.

CD4+CD25+ immunoregulatory T cells represent a unique lineage of thymic-derived cells that potently suppress both in vitro and in vivo effector T cell functions. There is mounting evidence that CD4+CD25+ T cells represent an important mechanism for the maintenance of self-tolerance (59–63), and they have been shown to prevent or limit experimentally induced organ-specific autoimmune diseases (1, 61, 64, 65). Several recent studies also demonstrated that removal of these cells from animals that normally do not develop autoimmune disease could result in autoimmunity (29, 66–67). In most mouse strains, this subset represents 7–10% of the CD4+ T cell subset, while Salomon et al. (1) reported that these cells are reduced in number and represent only 5–6% of the CD4+ T cells in NOD mice. In the present study, we found that both E2f1-deficient NOD and wild-type NOD mice have reduced numbers of CD4+CD25+ T cell subsets, and these T cell subsets were significantly reduced in spleen cells of N5 E2f1−/− mouse as compared with the wild-type NOD.E2f1+/−/+ mice (Fig. 8). These results reflect an earlier onset of IDDM and SS, along with an excessive number of mature CD4/CD8 single-positive thymocytes and peripheral T cells in 5-wk-old NOD.E2f1+/−/+ mice as compared with their wild-type NOD.E2f1+/−/+ mice (Figs. 1, 5, and 6) (1, 42, 68, 69). A mutation of the E2f1 molecule from normal standard NOD mice caused an inappropriate cell cycle distribution in thymocytes and peripheral spleen T lymphocytes. Th cell effector function such as cytokine (IFN-γ or IL-4) secretion is dependent on T cell proliferation (72–74). The increased production of IFN-γ observed in the present study is consistent with those reports and suggests that the increased number of inflammatory T cells in E2f1−/− mice may contribute to the development of autoimmune diseases by increasing T cell effector functions. We suggest that molecules such as E2f1, which can intervene in cell cycle control mechanisms, have a functional effect to restrict the development of early and increased onset of IDDM and SS in NOD mice. Furthermore, E2f1-deficient N5 (NOD.E2f1−/−) mice may be a useful model to study the functional role of the E2f1 molecule and other E2F molecules in IDDM and SS, as well as other dry mouth-type diseases.

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