Metabolic and Immunologic Consequences of Limited Adenosine Deaminase Expression in Mice*

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Adenosine deaminase (ADA; EC 3.5.4.4) deficiency in humans is an autosomal recessive genetic disorder that results in severe combined immunodeficiency disease. ADA-deficient mice generated by targeted gene disruption die perinatally, preventing postnatal analysis of ADA deficiency. We have recently rescued ADA-deficient fetuses from perinatal lethality by expression of an ADA minigene in the placentas of ADA-deficient fetuses, thus generating postnatal mice amenable to analysis of ADA deficiency. The minigene used also directed ADA expression to the forestomach postnatally, producing adult animals that lacked ADA enzymatic activity in all tissues outside the gastrointestinal tract. Mice with limited ADA expression exhibited profound disturbances in purine metabolism, including thymus-specific accumulations of deoxyadenosine and dATP, and inhibition of S-adenosylhomocysteine hydrolase in the thymus, spleen, and, to a lesser extent, the liver. Lymphopenia and mild immunodeficiency were associated with these tissue-specific metabolic disturbances. These mice represent the first genetic animal model for ADA deficiency and provide insight into the tissue-specific requirements of ADA.

Genetic defects in purine metabolism in humans result in serious metabolic disorders (1), often with tissue-specific phenotypes. A particularly striking example of this is adenosine deaminase (ADA) deficiency, which if untreated results in severe combined immunodeficiency disease (2). Although ADA deficiency was the first of the inherited immunodeficiencies for which the underlying genetic defect was identified (3), more than 25 years of subsequent research has not yielded a completely satisfactory explanation for the metabolic mechanisms which lead to the lymphoid specificity of the disease. However, available evidence suggests that the metabolic basis for ADA-deficient immunodeficiency is related to the physiological impact of the ADA substrates, adenosine and deoxyadenosine (Fig. 1) (2, 4). Adenosine functions as an extracellular signal transducer that mediates a variety of physiological effects by binding to adenosine receptors present on the surface of target cells (5). Deoxyadenosine behaves as a cytotoxic metabolite and is believed to provide the metabolic basis for ADA-deficient immunodeficiency (2). There are two mechanisms through which deoxyadenosine is thought to be lymphotoxic (Fig. 1). In one case, the accumulation of intracellular deoxyadenosine interferes with deoxynucleotide synthesis via its phosphorylation to dATP and subsequent inhibition of ribonucleotide reductase (6–10). The other mechanism involves inhibition of the enzyme S-adenosylhomocysteine (AdoHcy) hydrolase by deoxyadenosine (11). Inhibition of AdoHcy hydrolase in turn leads to the accumulation of AdoHcy which acts as an inhibitor of S-adenosylmethionine (AdoMet)-mediated transmethylation reactions (12). Although these pathways appear to be accessed in ADA-deficient cells, the mechanisms involved in selective T and B cell elimination are not fully understood.

Biological hallmarks of ADA deficiency in humans are consistent with these proposed mechanisms of deoxyadenosine cytotoxicity. They include increases in plasma adenosine and deoxyadenosine (13), elevations of deoxyadenosine in the urine, marked accumulations of dATP in erythrocytes (10, 14), and inhibition of erythrocyte AdoHcy hydrolase (12). A smaller population of ADA-deficient patients has been identified with late/ delayed-onset of combined immunodeficiency disease (15). These patients harbor mutations that allow for low levels of ADA enzymatic activity, resulting in less severe accumulations of ADA substrates and the persistence of some T and B cells. This supports the hypothesis that the severity of the disease relates to the severity of substrate accumulations. However, exactly how the disturbances in deoxynucleotide metabolism and AdoMet-mediated transmethylation lead to the immunodeficiency seen in the absence of ADA is still unclear. Furthermore, it has been difficult to correlate metabolic disturbances with specific tissues due to their inaccessibility in humans. Efforts to understand the metabolic and tissue-specific basis for the immunodeficiency would likely benefit from the development of an ADA-deficient animal model in which the metabolic effects associated with ADA deficiency can be easily studied in the relevant tissues.

Attempts to generate ADA-deficient mice have recently been conducted by two groups, producing animals with independent sites of gene disruption (16, 17). In both cases similar phenotypes were observed. Heterozygous matings produced Ada-null fetuses that died perinatally in association with severe liver damage, incomplete expansion of the lungs, and small intestine cell death (16, 17). Liver damage was evident by 16.5 days post-coitum and worsened through 18.5 days post-coitum, preceding death of the fetuses (16). This phenotype was accompanied by pronounced disturbances in purine metabolism (16, 17). The lymphoid organs of ADA-deficient fetuses and newborn pups were not largely affected, although there were minor...
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EXPERIMENTAL PROCEDURES

Mice and Histology—Mice heterozygous for the null Ada allele (m1/+), and hemizygous for the Ada minigene locus (Tg) were intercrossed to produce rescued mice that were homozygous for the null Ada allele (Tg, m1/m1) (21). Genotypes were determined by Southern blot analysis of genomic DNA obtained from tails at weaning (16, 19). Organs from aged-matched Tg, m1/+ and Tg, m1/m1 adults (9 weeks old) were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin according to standard procedures.

ADA Enzymatic Assay—Aged-matched wild type (+/+), and Tg, m1/m1 adult mice were sacrificed and tissues dissected into ice-cold phosphate-buffered saline (PBS). Tissue extracts were generated and ADA enzymatic activity measured in high-speed supernatants under saturating substrate conditions using a spectrophotometric assay (19). The decrease in absorbance at 265 nm resulting from the deamination of adenosine to inosine was continuously monitored in a Beckman DU-50 spectrophotometer, and the rate of inosine production was calculated at linearity. Specific activities are presented as nanomoles of adenosine converted to inosine per min per mg of protein.

Zymogram Analysis—When tails were biopsied at weaning for the determination of genotypes, a drop of blood was also collected to monitor ADA enzymatic activity by zymogram analysis. One volume of homogenization buffer (19) was added, and cells were lysed by several rounds of freeze-thawing. Equal amounts of protein (20 μg) were subjected to zymogram analysis to visualize ADA and purine nucleoside phosphorlyase (PNP) enzymatic activities (18).

Analysis of Nucleosides and Nucleotides—Tissues from aged-matched Tg, m1/+, and Tg, m1/m1 mice 9–13 weeks of age were dissected into ice-cold PBS and then quick-frozen in liquid nitrogen for extraction and analysis of nucleosides (21, 22). The HPLC system consisted of two Contrametric III pumps controlled by a GM 4000 gradient programmer, a constant wavelength UV monitor, and a CI-10B integrator (LDC Analytical). Separation was through a reversed-phase Customsil ODS column (4.6 × 254 mm) with a 20-mm ODS precolumn (Custom LC Inc.). The mobile phase was 0.2 M NH4H2PO4 (pH 5.1) with a superimposed methanol gradient. Flow rate was 1 ml/min and the injection volume 200 μl. Absorbance was continuously monitored at a wavelength of 254 nm, and peaks were identified and quantitated based on co-retention of known amounts of external standards (Sigma). Peaks of interest were verified by enzymatic shift assay. Whole blood was obtained by cardiac puncture, and serum and cellular components were separated by centrifugation at 2,600 rpm for 10 min at 4 °C. Serum was processed for nucleoside levels, and packed erythrocytes were analyzed for nucleotides.

Nucleotides were extracted with 20 volumes of 60% methanol at 20 °C overnight (21, 23). Samples were filtered through Millex-GV4 membrane filters (Millipore) and stored at −20 °C until analyzed by HPLC. The HPLC conditions were as described above except the co-retaining mobile phase consisted of 0.5 M NH4H2PO4 (pH 6.5), 2 M tetra-butylammonium hydroxide, and 7% acetonitrile, and the flow was isocratic.

AdoHcy Hydrolase Enzymatic Assay—Aged-matched Tg, m1/+, +/+ and Tg, m1/m1 adult mice were sacrificed and tissues dissected into ice-cold PBS. Tissue extracts were generated according to established procedures, and extracts containing 0.4 μg of protein (liver) or 2.0 μg of protein (other tissues) were incubated at 37 °C for 1 h in a reaction mixture containing 157 μM [8-14C]adenosine, 51 μCi/mmol, 20 μM α,β-deoxycoformycin (11). After incubation, aliquots (7 μl) were spotted onto thin layer plates, and chromatography was carried out using butanol-1/methanol/H2O/NH4OH (60:20:20:1, v/v). Relative amounts of 14C detected in substrate and products were quantitated by phosphorimaging. Specific activities are given as nanomoles of [14C]AdoHcy formed per min per mg of protein.

Flow Cytometry of Leukocyte Populations—The following monoclonal antibodies used for flow cytometric analysis were obtained from Pharmingen, Inc. (San Diego, CA) as fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugates: anti CD3-e, clone 145–2C11; anti CD4, clone RM4–5; anti-CD8a, clone 53–6.7; anti-CD45R/B220, clone RA3–6B2; anti-Gr-1/myeloid differentiation antigen, clone RB6–8C5; anti-CD11b (Mac-1), clone M1/70. Phenotyping of leukocyte subpopulations in thymuses and spleens was conducted by direct two-color analysis with FITC- and PE-conjugated antibodies on an EPIC Profile Analyzer, according to manufacturer's instructions (Pharmingen, Inc.). Thymuses and spleens were excised from Tg, m1/+ and Tg, m1/m1 aged-matched mice.
ADA enzymatic activity is restricted to the gastrointestinal tract, principally the forestomach. To confirm this expected pattern of ADA minigene expression, ADA enzymatic activity was measured in various tissues from adult rescued mice. Circulating ADA enzymatic activity was assayed by zymogram analysis of blood collected from mice during tail biopsies at weaning (Fig. 2A). ADA was readily detected in wild type (+/+) and heterozygous (m1/+), but was not detected in mice homozygous for the null Ada allele and carrying the ADA minigene locus (Tg, m1/m1). In addition to providing evidence that these animals were deficient in circulating ADA enzymatic activity, this procedure provided a sensitive and rapid means of identifying rescued mice. Homogenates from various tissues collected from +/+ and Tg, m1/m1 adult mice were assayed for ADA enzymatic activity. As expected, high levels of ADA minigene expression, similar to those of native ADA, were detected in the forestomach of Tg, m1/m1 mice (Fig. 2B). Lower levels of ADA minigene expression were detected elsewhere in the gastrointestinal tract (Fig. 2B) but no expression was detected in the thymus, spleen, or other organs examined (Fig. 2C). Therefore, postnatal ADA expression from the minigene locus was restricted to the gastrointestinal tract, principally the forestomach.

Purine Metabolism Is Altered in Mice with Limited ADA Expression—ADA deficiency in humans and murine fetuses is associated with profound disturbances in purine metabolism (2, 16) which are thought to provide the metabolic basis for immunodeficiency and perinatal lethality, respectively. Adenine nucleoside and nucleotide levels were measured in Tg, m1/m1 mice to determine the metabolic consequences associated with limited ADA expression in rescued mice. Acid-soluble extracts were made from various tissues from Tg, m1/+ and Tg, m1/m1 mice and adenine nucleosides were identified and quantitated using reversed phase HPLC. Representative HPLC profiles from thymuses are shown in Fig. 3 and indicate substantial increases in adenosine and deoxyadenosine in Tg, m1/m1 animals. Disturbances in purine metabolism were detected in all tissues examined (Fig. 4). Inosine levels were lower in the thymus, spleen, liver, and kidney of Tg, m1/m1 mice (Fig. 4A). Adenosine levels were elevated in all tissues examined (Fig. 4B).
4B), with the greatest elevation occurring in the liver (9.4-fold) and thymus (7.3-fold). Deoxyadenosine accumulated in a thymus-specific manner, with levels increasing approximately 800-fold, from undetectable levels (<0.001 nmol/mg protein) in Tg, m1+/m1 thymuses, to 0.79 nmol/mg protein, in Tg, m1/m1 thymuses (Fig. 4C). These data demonstrate that there are profound disturbances in the levels of ADA substrates and products in mice with limited ADA expression. Moreover, there were thymus-specific elevations in the levels of deoxyadenosine found in Tg, m1/m1 mice, suggesting this lymphotoxic molecule may elicit a harmful effect on the immune system of these mice.

Deoxyadenosine is toxic to cells through mechanisms that include its phosphorylation to dATP (9) and subsequent inhibition of ribonucleotide reductase (Fig. 1) (6). The pronounced accumulation of deoxyadenosine in thymuses of Tg, m1/m1 mice prompted us to investigate adenine deoxynucleotide pools in these mice. Nucleotides were extracted from the thymus, spleen, liver, and erythrocytes of Tg, m1+/m1 and Tg, m1/m1 adult mice, and ATP and dATP were analyzed and quantitated by ion pairing reversed phase HPLC. ATP was readily detected in all samples (Table I), with slight elevations in ATP occurring in the thymus, spleen, and erythrocytes of Tg, m1/m1 mice. dATP was not detected at a lower limit of detection of ≤0.01 nmol/mg protein in any tissue examined except for the thymus of Tg, m1/m1 mice, where it increased over 50-fold. Consistent with these observations, dAMP, an intermediate in the phosphorylation of deoxyadenosine to dATP, was markedly increased in the thymus of Tg, m1/m1 mice (Table I, also see Fig. 3). The thymus and, to a lesser extent, the spleen were the only tissues in Tg, m1+/m1 mice in which dAMP was detected, albeit at low levels. These data suggest that deoxyadenosine accumulating in the thymus of Tg, m1/m1 mice is phosphorylated to dATP, providing compelling evidence for deoxyadenosine cytotoxicity in this organ.

AdoHcy Hydrolase Enzymatic Activity Is Inhibited in Tissues of Rescued Mice—Another metabolic consequence of ADA deficiency is the inhibition of AdoHcy hydrolase (11), which leads to the accumulation of AdoHcy and the disruption of cellular transmethylation reactions involving AdoMet (Fig. 1) (12). To begin to assess the involvement of this pathway in adult mice with limited ADA expression, we measured AdoHcy hydrolase enzymatic activity in tissues of adult Tg, m1+/m1 and Tg, m1/m1 mice (Fig. 5). AdoHcy hydrolase enzymatic activity was detected in all tissues examined but in varying amounts (Fig. 5). AdoHcy enzymatic activity in the liver was at least 7-fold higher than the moderate levels measured in the small intestine, kidney, thymus, and spleen. The lowest levels of activity measured were in erythrocytes, brain, heart, and lung (data not shown). AdoHcy hydrolase enzymatic activity was substantially inhibited in the thymus and spleen of Tg, m1/m1 mice. Inhibition was also seen in the livers of these animals but to a lesser extent. There was no significant inhibition found in the small intestine or kidney of Tg, m1/m1 adult mice. These data suggest that AdoHcy metabolism is subject to lymphoid-specific disturbances in mice with limited ADA expression.

Mice with Limited ADA Expression Exhibit Lymphopenia and Partial Immunodeficiency—Thymus-specific accumula-

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**Fig. 3.** HPLC chromatographic profiles of nucleosides isolated from the thymus of Tg, m1+/m1 (A) and Tg, m1/m1 (B) mice. Nucleosides were extracted from thymuses and analyzed by reversed phase HPLC. Profiles represent runs of equal protein concentrations from the thymus of Tg, m1+/m1 and Tg, m1/m1 littermates. Peaks identified and quantitated include Ino, inosine; Ado, adenosine; dAdo, deoxyadenosine; dAMP, deoxyadenosine monophosphate.

**Fig. 4.** Levels of purine nucleosides and nucleotides in tissues from Tg, m1+/m1 and Tg, m1/m1 mice. Tissues examined include thymus, spleen, liver, kidney, and serum. Nucleosides were extracted, analyzed, and quantitated by reversed phase HPLC (Fig. 3). Concentrations are given as nmol/mg protein for mean values ± S.E.; n = 3 for each genotype; ND, not detected at a lower limit of ≤0.001 nmol/mg protein. A, inosine; B, adenosine; C, deoxyadenosine.
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**Table I**

Adenine nucleotide and deoxynucleotide concentrations in adult tissues

| Organ      | Genotype  | ATP     | dATP     | dAMP     |
|------------|-----------|---------|----------|----------|
| Thymus     | Tg, m1/+  | 5.28 ± 1.56 | ND* | 0.88 ± 0.42 |
|            | Tg, m1/m1 | 3.38 ± 1.10 | ND      | 0.03 ± 0.01 |
| Spleen     | Tg, m1/+  | 6.70 ± 2.11 | 0.50 ± 0.24 | 8.99 ± 3.02 |
|            | Tg, m1/m1 | 6.30 ± 1.48 | ND | 0.05 ± 0.03 |
| Liver      | Tg, m1/+  | 3.87 ± 0.56 | ND | ND |
|            | Tg, m1/m1 | 3.23 ± 0.96 | ND | ND |
| Erythrocytes | Tg, m1/+ | 6.96 ± 1.97 | ND | ND |
|            | Tg, m1/m1 | 9.21 ± 4.85 | ND | ND |

* ND, not detected at a level of ≤0.01 nmol/mg protein.

**DISCUSSION**

ADA-deficient fetuses die perinatally (16, 17), suggesting ADA is essential for fetal survival, but preventing our ability to assess the metabolic and immunologic consequences of ADA deficiency in adult mice. We were able to rescue these fetuses by restoring ADA to deficient placentas using an ADA minigene driven by Ada gene regulatory elements known to target the primary organ affected in ADA-deficient fetuses die perinatally (16, 17), suggesting ADA is essential for fetal survival, but preventing our ability to assess the metabolic and immunologic consequences of ADA deficiency in adult mice. We were able to rescue these fetuses by restoring ADA to deficient placentas using an ADA minigene driven by AdoHcy hydrolase enzymatic activity determined. Values are given as nanomoles of substrate converted per min per mg protein for means ± S.E.; n = 4; *, p < 0.01; **, p < 0.005; ***; p < 0.001.

To evaluate leukocyte subpopulations, flow cytometry was performed on thymus and spleen cell populations from aged-matched Tg, m1/+ and Tg, m1/m1 mice using antibodies to the cell surface antigens, CD3, CD4, CD8, CD45R, GR-1, and CD11b. Results from these analyses are shown in Table II. Although the thymus of Tg, m1/m1 mice showed a large reduction in size and lymphoid cell number, there were no significant differences in the distribution of leukocyte subpopulations. There was, however, a slight reduction in CD4, CD8 double positive cells in Tg, m1/m1 thymuses. Leukocyte subpopulations were significantly altered in Tg, m1/m1 spleens. The most significant change was a decrease in spleen T cell populations. Cells positive for the T cell antigen CD3 were reduced from 45% in Tg, m1/+ spleens to 31% in Tg, m1/m1 spleens. Among the T cell populations, the greatest reduction was in CD4-positive cells (p < 0.001), with a decrease from 32% in Tg, m1/+ spleens to 17% in Tg, m1/m1 spleens. There was also a slight reduction in CD8-positive T cells. Significant increases in B cells (CD45R+ positive) from 45 to 56% were observed, as well as increases in granulocytes (GR-1-positive, 6–9%) and macrophages (CD11b-positive, 8–14%). These results indicate that Tg, m1/m1 mice exhibit alterations in leukocyte populations with the greatest effect being a CD4 lymphopenia.

**Fig. 5.** AdoHcy hydrolase enzymatic activity in tissues from Tg, m1/+ and Tg, m1/m1 mice. The liver, small intestine (si), kidney, thymus, spleen, and erythrocytes (rbc) of aged matched adult Tg, m1/+ and Tg, m1/m1 mice were harvested and AdoHcy hydrolase enzymatic activity determined. Values are given as nanomoles of substrate converted per min per mg protein for means ± S.E.; n = 4; *, p < 0.01; **, p < 0.005; ***; p < 0.001.

**TABLE II**

Adenine nucleotide and deoxynucleotide concentrations in adult tissues

| Organ       | Genotype  | ATP     | dATP     | dAMP     |
|-------------|-----------|---------|----------|----------|
| Thymus      | Tg, m1/+  | 5.28 ± 1.56 | ND* | 0.88 ± 0.42 |
|             | Tg, m1/m1 | 3.38 ± 1.10 | ND      | 0.03 ± 0.01 |
| Spleen      | Tg, m1/+  | 6.70 ± 2.11 | 0.50 ± 0.24 | 8.99 ± 3.02 |
|             | Tg, m1/m1 | 6.30 ± 1.48 | ND | 0.05 ± 0.03 |
| Liver       | Tg, m1/+  | 3.87 ± 0.56 | ND | ND |
|             | Tg, m1/m1 | 3.23 ± 0.96 | ND | ND |
| Erythrocytes | Tg, m1/+ | 6.96 ± 1.97 | ND | ND |
|             | Tg, m1/m1 | 9.21 ± 4.85 | ND | ND |

* ND, not detected at a level of ≤0.01 nmol/mg protein.

**DISCUSSION**

ADA-deficient fetuses die perinatally (16, 17), suggesting ADA is essential for fetal survival, but preventing our ability to assess the metabolic and immunologic consequences of ADA deficiency in adult mice. We were able to rescue these fetuses by restoring ADA to deficient placentas using an ADA minigene driven by Ada gene regulatory elements known to target expression to the placenta prenatally and the fetus postnatally (21, 19). In accordance with this, rescued adult mice homozygous for the null Ada allele and containing the ADA minigene locus did not express ADA in any tissue outside the gastrointestinal tract. Profound disturbances in purine metabolism were observed in these mice, with adenosine accumulating in all tissues examined, whereas deoxyadenosine and dATP
accumulated in a thymus-specific manner. AdoHcy hydrolase, a key enzyme in transmethylation reactions involving AdoMet, was markedly inhibited in the thymus, spleen, and to a lesser extent the liver but not in other tissues examined. The status of the immune system in these mice was examined, and a major reduction in the size of the thymus and spleen was found. In addition, changes in leukocyte subpopulations were observed with the greatest effect being a reduction in CD4-positive T lymphocytes found in the spleen. Mitogen stimulation assays showed a decrease in the responsiveness of T and B lymphocytes suggesting a partial immunodeficiency. These findings suggest that the lymphopenia and partial immunodeficiency in mice with limited ADA expression likely result from tissue-specific disturbances in purine metabolism.

Our efforts have generated the first genetic animal model for studying the metabolic and immunologic consequences of ADA deficiency in postnatal mice. This has allowed us to investigate the metabolic impact of ADA deficiency in tissues not easily accessible in ADA-deficient humans. The most striking observation was the accumulation of deoxyadenosine and deoxyadenosine nucleotides in the thymus. Thymus-specific accumulation of deoxyadenosine supports the hypothesis that deoxyadenosine lymphotoxicity is involved in the immune phenotype found in rescued mice. Moreover, thymus-specific accumulation of dAMP and dATP provide evidence that deoxyadenosine is readily phosphorylated. This feature is consistent with in vitro studies suggesting that inhibition of ribonucleotide reductase by dATP is involved with phenotypes resulting from ADA deficiency (Fig. 1) (6–10). Interestingly, dAMP was detected at low levels in the thymus of control animals. This may be a result of DNA breakdown from massive apoptosis which occurs in the thymus as a natural part of intrathymic T cell development and selection (24). Endogenous ADA levels in the thymus are high (20, 25, 26), most likely to handle this production of deoxyadenosine from dAMP. This is supported by the marked thymus-specific accumulation of deoxyadenosine and deoxynucleotides in mice lacking ADA in their thymuses. Recent studies have suggested that immature T lymphocytes are sensitive to the accumulation of deoxyadenosine through mechanisms involving p53-dependent apoptosis (27). It is possible that similar mechanisms are involved in the T cell lymphopenia observed in rescued mice and that T lymphocytes are depleted during intrathymic development. The animals generated here provide a valuable in vivo genetic model for learning

![Fig. 6. Reduction in lymphoid organ size. Spleen (A) and thymus (B) from 9-week-old Tg, ml/+ and Tg, ml/ml littermates. A similar reduction in organ size was observed in all animals examined, n = 26. Cross-sections through Tg, ml/+ (C) and Tg, ml/ml (E) adult thymuses stained with hematoxylin and eosin. m, medullary region; c, cortical region; arrow, Hassal’s corpuscle. Cross-sections through Tg, ml/+ (D) and Tg, ml/ml (F) adult spleens stained with hematoxylin and eosin. w, white pulp; r, red pulp; bar = 100 μm.]
more about how disturbances in deoxynucleotide metabolism influence the growth and development of T and B lymphocytes.

Another proposed mechanism of immunodeficiency associated with ADA deficiency is the inhibition of methylation reactions involving AdoMet (11, 12). The product of such methylation reactions is AdoHcy, which is hydrolyzed to adenosine and homocysteine by AdoHcy hydrolase (Fig. 1) (12, 28). Inhibition of AdoHcy hydrolase can lead to the accumulation of AdoHcy, which functions as a competitive inhibitor of many transmethylation reactions critical to cellular function. Inhibition of AdoHcy hydrolase has been associated with ADA deficiency in humans (12) and in ADA-deficient perinatal mice (17). The ability to examine individual tissues in mice with limited ADA expression revealed a correlation between the inhibition of AdoHcy hydrolase activity and the immune phenotype observed. Furthermore, inhibition of AdoHcy hydrolase enzymatic activity in the thymus together with the thymus-specific accumulations in deoxyadenosine are consistent with the inactivation of AdoHcy hydrolase by deoxyadenosine (11). However, this does not appear to be the case for AdoHcy hydrolase inhibition in other tissues that did not accumulate deoxyadenosine, such as the spleen and liver. There is evidence that AdoHcy hydrolase is inhibited by other nucleosides and nucleotides (29, 30). In particular, patients with PNP and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency show decreases in erythrocyte AdoHcy hydrolase enzymatic activity (30, 31). Since deoxyadenosine is not elevated in these patients, AdoHcy hydrolase inhibition is likely due to other mechanisms. For example, inosine, whose levels can be elevated in the absence of both PNP and HGPRT enzymatic activity, has been shown to inactivate purified AdoHcy hydrolase, as well as in HGPRT-deficient lymphoblasts (30). Therefore, AdoHcy hydrolase inhibition in the spleen and liver of Tg, m1/m1 mice may result from purine metabolic disturbances other than deoxyadenosine accumulation. It is likely that these mice will provide a novel means of studying the mechanism(s) of AdoHcy hydrolase inactivation in animals and humans with genetic disorders of purine metabolism.

The other ADA substrate, adenosine, acts as an extracellular signal, engaging cell surface receptors to elicit an array of cellular functions (Fig. 1) (5). Adenosine has been shown to induce apoptosis in T lymphocytes in a receptor-mediated manner (32). Accumulation of adenosine was detected in all tissues of rescued mice examined; however, no obvious phenotype was observed in tissues outside the immune system, suggesting that increased adenosine concentrations are not overly harmful. Although most evidence suggests deoxyadenosine is the primary lymphotoxic substrate in ADA deficiency, it cannot be ruled out that disruption of adenosine signaling in the thymus and spleen may be involved in the phenotype observed. Investigations into adenosine receptor distribution and engagement in these mice will help to clarify the involvement, if any, of adenosine signaling in the immune phenotype observed.

Disturbances in purine metabolism and subsequent immune phenotypes have been produced in mice treated with the ADA inhibitor 2’-deoxycoformycin (33–36); however, the extent and duration of ADA inhibition in these pharmacological models is variable, making it difficult to accurately assess the metabolic and immunologic effects of ADA deficiency. Because of the genetic nature of the experiments conducted here, the impact of continuous ADA deficiency was made possible. Consequently, tissue-specific disturbances in deoxyadenosine were observed which correlate to partial immunodeficiency. The most striking effect on the immune system was the decrease in size and total lymphoid cell number in the thymus and spleen, suggesting lymphopenia. The medulla of ADA-deficient thymuses was considerably smaller in proportion to the cortex as compared with what is seen in normal mice. Given that the medulla harbors mature CD4 and CD8 single positive cells, one might expect a decrease in the percentage of this population ADA-deficient thymuses. This was not the case, however; there was a significant decrease in the percentages of CD4 and CD8 cells found in the spleen. The reason for this is not clear; however, it may reflect the inability of double positive cells to fully mature and emigrate out of the thymus. This feature has been noted pre-

**TABLE II**

| Cell surface antigen | Tg, m1/+ | Tg, m1/m1 | Tg, m1/+ | Tg, m1/m1 |
|----------------------|---------|---------|---------|---------|
|                      | Thymus  | Spleen  | Thymus  | Spleen  |
| CD3                  | 38.7 ± 6.2 | 36.7 ± 15.8 | 45.0 ± 2.5 | 31.0 ± 2.5* |
| CD4                  | 67.7 ± 6.0  | 67.0 ± 6.0  | 31.7 ± 1.2  | 17.0 ± 1.2b  |
| CD8                  | 59.3 ± 18.9 | 53.3 ± 12.4 | 10.3 ± 1.2  | 7.3 ± 0.9    |
| CD4 + CD8            | 80.7 ± 6.3  | 74.0 ± 8.9  | 1.9 ± 0.1   | 0.5 ± 0.3    |
| CD45R                | 1.0 ± 0.5   | 1.0 ± 0.5   | 44.7 ± 2.9  | 56.0 ± 1.5a  |
| GR-1                 | 0.9 ± 0.3   | 3.0 ± 2.1   | 5.6 ± 0.8   | 8.9 ± 1.1c   |
| CD11b                | 3.2 ± 1.2   | 1.9 ± 0.1   | 8.3 ± 0.9   | 13.6 ± 4.5   |

*Significantly different from control at a value of p < 0.02.

**Fig. 7.** Reduction in total lymphoid cells in thymuses and spleens of adult Tg, m1/m1 mice. Data are given as total lymphoid cells (in millions) ± S.E.; spleens, n = 9 for each genotype; thymus, n = 7 for each genotype; *, significant with p < 0.05; **, significant with p = 0.01.

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that strengthen the hypothesis that deoxyadenosine accumulation in the thymus is responsible for the immune phenotype associated with ADA deficiency. Furthermore, we provide in vivo evidence that disturbances in both deoxynucleotide metabolism and AdoMet-mediated transmethylation reactions are involved. These mice will be a useful genetic model for assessing the relative involvement of each of these pathways in the T and B cell lymphopenia associated with ADA deficiency.

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