Adenosine Deaminase-deficient Mice Generated Using a Two-stage Genetic Engineering Strategy Exhibit a Combined Immunodeficiency*

(Received for publication, October 9, 1997, and in revised form, December 1, 1997)

Michael R. Blackburn‡, Surjit K. Datta, and Rodney E. Kellem.§

From the Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030 and the Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School, Houston, Texas 77030

Adenosine deaminase (ADA) deficiency in humans leads to a combined immunodeficiency. The mechanisms involved in the lymphoid specificity of the disease are not fully understood due to the inaccessibility of human tissues for detailed analysis and the absence of an adequate animal model for the disease. We report the use of a two-stage genetic engineering strategy to generate ADA-deficient mice that retain many features associated with ADA deficiency in humans, including a combined immunodeficiency. Severe T and B cell lymphopenia was accompanied by a pronounced accumulation of 2’-deoxyadenosine and dATP in the thymus and spleen, and a marked inhibition of S-adenosylhomocysteine hydrolase in these organs. Accumulation of adenosine was widespread among all tissues examined. ADA-deficient mice also exhibited severe pulmonary insufficiency, bone abnormalities, and kidney pathogenesis. These mice have provided in vivo information into the metabolic basis for the immune phenotype associated with ADA deficiency.

Genetic defects in purine metabolism in humans result in serious metabolic disorders, often with pronounced tissue-specific phenotypes (1). A striking example of this is adenosine deaminase (ADA) deficiency, which results in impaired lymphoid development and a severe combined immunodeficiency disease (SCID) (2). ADA-deficient SCID was the first of the inherited immunodeficiencies for which the underlying molecular defect was identified (3); however, despite over 20 years of subsequent research, a satisfactory explanation for the lymphoid specificity of this metabolic disease has not emerged. This is largely due to the inaccessibility of human tissue for detailed phenotypic and metabolic analysis and the absence of an animal model which retains features of ADA deficiency in humans. The availability of a genetic animal model for ADA deficiency would make possible a wide range of biochemical and immunological experiments that are not permissible with humans. Additional interest in ADA deficiency stems from recent attempts to use novel therapeutic strategies, including enzyme therapy (4) and gene therapy (5, 6), to treat the condition in humans. Although the results of these therapeutic approaches are encouraging, unexpected outcomes have raised numerous important questions regarding the efficacy of specific treatment protocols (4, 7). The pace with which new enzyme and gene therapy protocols can be tested would be greatly increased by the availability of an animal model for ADA deficiency.

Successful attempts to generate ADA-deficient mice were initially reported by two groups (8, 9), resulting in animals with independent sites of Ada gene disruption. In each case, a similar phenotype was observed. ADA-deficient fetuses died perinatally due to severe liver damage (8, 9). This phenotype was accompanied by profound disturbances in purine metabolism, including marked increases in the ADA substrates adenosine and 2’-deoxyadenosine. Both adenosine and 2’-deoxyadenosine are biologically active purines that can have profound impact on cellular physiology. Adenosine is an extracellular signaling molecule that binds G-protein-coupled receptors on the surface of target cells to elicit a wide variety of physiological responses (10) including T lymphocyte cell death (11, 12). 2’-Deoxyadenosine is a cytotoxic metabolite that can kill cells through mechanisms that include disturbances in deoxynucleotide metabolism (13, 14) and the inhibition of cellular transmethylations reactions (15, 16). ADA-deficient fetuses exhibited evidence for both of these mechanisms of 2’-deoxyadenosine cytotoxicity, in that levels of the 2’-deoxyadenosine metabolite, dATP, were markedly elevated, and the enzyme S-adenosylhomocysteine (AdoHcy) hydrolase was inhibited (8, 9). These metabolic disturbances are thought to contribute to the liver damage and subsequent death of ADA-deficient fetuses.

The perinatal death of ADA-deficient fetuses precluded our ability to assess the consequences of ADA deficiency in postnatal animals. In the current report, we describe the use of a transgenic strategy whereby we rescued ADA-deficient fetuses from perinatal lethality by restoring Ada expression to trophoblast cells. This provided us with postnatal animals that were completely ADA-deficient. These ADA-deficient mice retain many features seen in ADA-deficient humans, in particular a severe lymphopenia and combined immunodeficiency. In addition, ADA-deficient mice develop severe pulmonary insufficiency, and bone and kidney abnormalities were detected. The ability to examine metabolic disturbances in a variety of tissues of ADA-deficient mice revealed a widespread accumulation of adenosine, whereas 2’-deoxyadenosine accumulated in a predominantly lymphoid-specific manner. AdoHcy hydrolase inhibition and dATP accumulation were greatest in the thymus and spleen of ADA-deficient mice. These genetically engineered

*This work was supported by National Institutes of Health Grants DK46207 and HD34130 and by a grant from the R. A. Welch Foundation.
‡Supported by National Institutes of Health Postdoctoral Fellowship HD97843.
§To whom correspondence should be addressed. Current address: Dept. of Biochemistry and Molecular Biology, University of Texas-Houston Medical School, 6431 Fannin St., Houston, TX 77030; Tel.: 713-500-6124; Fax: 713-500-0652; E-mail: rkellem@utming.med.uth.tmc.edu.

The abbreviations used are: ADA, adenosine deaminase; SCID, severe combined immunodeficiency disease; AdoHcy, S-adenosylhomocysteine; kb, kilobase pair(s); RBC, red blood cell.
ADA-deficient mice have thus provided novel in vivo information into the metabolic basis for the immune phenotype associated with ADA deficiency.

EXPERIMENTAL PROCEDURES

Generation and Characterization of Mice—An ADA minigene (18) was fused to a 2.0-kb BamHI to EagI genomic fragment in the 5′-flanking region of the murine Ada gene that contained a 770-base pair trophoblast regulatory element (17). This construct was purified and used to generate transgenic mice (18). Genomic DNA was isolated from tails at postnatal days 15–22, digested with BamHI, and hybridized with the internal 3.5-kb probe shown in Fig. 1C (5, 18). ADA transcript levels in placentas and fetuses were monitored by RNase protection assay. ADA enzymatic activity and zymogram analysis was conducted as described previously (18, 19). Mice were housed in microisolator cages in a colony free of common murine viruses and pathogens.

Histological Analysis—Tissues of interest were dissected from control and ADA-deficient mice on postnatal day 15. Tissues were rinsed in ice-cold phosphate-buffered saline and then fixed overnight in 4% paraformaldehyde in phosphate-buffered saline at 4 °C. Tissues were then dehydrated, cleared, and embedded in paraffin according to established protocols. Sections 7 μm in thickness were collected from each sample and stained with hematoxylin and eosin using a Rapid Chrome staining kit (Shandon). Photographs of sections were generated using an Olympus BX60 microscope with bright field illumination. Analysis of bone using alizarin red and azure blue was conducted according to established protocols.

Immunology—Determination of lymphoid cell counts in thymuses and spleens were conducted as described previously (19). For examination of peripheral lymphocytes, 200 μl of blood was collected from the tail vein of mice directly into EDTA-coated microcentrifuge tubes. Complete blood counts were determined using an H1 analyzer (Technicon Instruments Corp.). For determination of serum immunoglobulins, whole blood was collected by cardiac puncture, allowed to set on ice for 15 min and then centrifuged for 10 min at 2000 × g at 4 °C. Serum was then removed and stored at −70 °C until analyzed. Serum immunoglobulin levels were determined using a single radioimmunodiffusion assay (Charles River Laboratories). Direct two-color analysis with antibodies against the cell surface antigens CD3, CD4, CD8, TCR αβ, IgM, and B220 was performed according to established methods (19).
Nucleoside and Nucleotide Analysis and Determination of S-Adenosylhomocysteine Hydrolase Enzymatic Activity—Tissues were quickly removed from control or ADA-deficient mice and frozen in liquid nitrogen, and nucleosides and nucleotides were extracted and analyzed according to established procedures (18, 20, 21). AdoHcy hydrolase enzymatic activity was determined in freshly prepared tissue extracts according to established procedures (19).

RESULTS

Generation of ADA-deficient Mice Using a Two-stage Genetic Engineering Strategy—Previous studies suggest that Ada expression in trophoblast cells of the placenta is critical for fetal development in the mouse (18, 22). Thus, to generate completely ADA-deficient postnatal mice, an ADA minigene (Fig. 1A) that targeted expression specifically to the trophoblast lineage (Fig. 1B) was introduced onto the ADA-deficient background. This was accomplished by intercrossing mice carrying the trophoblast-specific ADA minigene (Tg) with mice heterozygous for the null Ada allele (m1/+). Subsequent intercrosses yielded litters that contained mice harboring the ADA minigene (Tg) that were also homozygous for the null Ada allele (m1/m1) (Fig. 1D). Given that the regulatory elements used targeted Ada expression only to trophoblasts, once born, and with the loss of the placenta, Tg-m1/m1 mice should lack ADA enzymatic activity. To monitor Ada expression in rescued mice, control and Tg-m1/m1 mice were sacrificed on postnatal day 15 and ADA enzymatic activity determined in various tissue extracts (Fig. 1E). ADA enzymatic activity was not observed in any of the tissues examined in Tg-m1/m1 mice. Therefore, expression in trophoblast cells alone was sufficient to rescue ADA-deficient fetuses from perinatal lethality, and provide postnatal mice that were ADA-deficient.

ADA-deficient Mice Develop Severe Lymphopenia and Combined Immunodeficiency—ADA-deficient humans fail to thrive and die within the first few months of life if not properly diagnosed and treated (2). ADA-deficient mice generated by trophoblast rescue also fail to thrive and die by approximately 3 weeks of age. Nevertheless, these mice provided us the opportunity to directly assess the effect of ADA deficiency on the thymus and spleen, critical immune organs that are not accessible for analysis in ADA-deficient humans. The status of the immune system in ADA-deficient mice was assessed between postnatal days 15 and 17 prior to a severe decline in health to minimize the influence of physiologic stress on the immune phenotype. Gross examination of the thymus and spleen in ADA-deficient mice revealed a substantial decrease in organ size that corresponded with a greater than 50% reduction in organ-to-body weights (data not shown). Histological analysis revealed a decrease in the size of the thymus (Fig. 2b) and spleen (Fig. 2f) of ADA-deficient mice. Examination at a higher magnification showed that there was a decrease in cortical-medullary demarcation in ADA-deficient thymuses, and...
Hassell's corpuscles were not found (Fig. 2d). Examination of spleens revealed a decrease in the number of red blood cells found in the red pulp, and few megakaryocytes were observed (Fig. 2h). Consistent with the decrease in organ size, lymphoid cell counts were substantially reduced in the ADA-deficient thymus (8-fold) and spleen (3-fold) (Fig. 3A). Lymphopenia was also seen in the peripheral circulation where ADA-deficient mice contained one-third the number of peripheral lymphoid cells seen in control mice (Fig. 3B). Serum immunoglobulin levels were measured to determine if the humoral branch of the immune system was affected by ADA deficiency. Total serum immunoglobulin levels were reduced 3-fold in ADA-deficient mice (Fig. 3C), suggesting an immunodeficiency. Because mice were analyzed prior to weaning, it is likely that maternal IgG accounts for most and possibly all of the serum immunoglobulins (23). Therefore, the ability of ADA-deficient mice to produce immunoglobulins is likely to be severely reduced. These data demonstrate that ADA-deficient mice exhibit a combined T and B cell lymphopenia and immunodeficiency.

It has not been possible to assess the distribution of lymphocyte populations in the immune organs of ADA-deficient humans. To determine whether genetic ADA deficiency leads to alterations in thymic cell populations, flow cytometry was performed on cellular populations from the thymuses of ADA-deficient mice. There were substantial differences in the distribution of thymocytes bearing cell surface antigens specific for different stages of T lymphocyte development. In ADA-deficient thymuses, there was a significant increase in the percentage of CD4\(^+\)CD8\(^-\) double-negative immature thymocytes, whereas there was a decrease in the percentage of CD4\(^+\)CD8\(^+\) double-positive thymocytes and CD4\(^-\)CD8\(^+\) single-positive thymocytes (Fig. 4A). These data suggest that developing thymocytes at the transition from the double-negative to double-positive stage are sensitive to the consequences of ADA deficiency.

Lymphoid cells from ADA-deficient spleens were examined to determine whether there were alterations in mature lymphoid populations. There was a substantial decrease in the percentage of mature CD4\(^+\) and CD8\(^+\) T lymphocytes in ADA-deficient spleens (Fig. 4B). An increase in granulocytes and macrophages, eosinophilia, and anemia were also noted in ADA-deficient mice (data not shown), whereas there was not a significant difference in the distribution of splenic cells harboring the B lymphocyte markers IgM or B220 (Fig. 4B). The decrease in the percentages of CD4\(^+\) and CD8\(^-\) T lymphocytes, together with the general T and B cell lymphopenia and hypogammaglobulinemia, suggest that ADA-deficient mice exhibit a combined immunodeficiency.

ADA-deficient Mice Exhibit Severe Disturbances in the Levels of the ADA Substrates Adenosine and 2'-Deoxyadenosine—Metabolic disturbances associated with ADA deficiency in humans have only been monitored in fluid and cellular components that are accessible, such as plasma, serum, and urine (2, 24, 25). The levels of adenosine and 2'-deoxyadenosine are readily detected in these samples; however, little to nothing is known with regard to the metabolic disturbances in tissues of ADA-deficient individuals. Monitoring metabolic disturbances in the tissues of ADA-deficient mice, in particular immunologic tissues, would provide important information into the mechanism of the immunodeficiency associated with ADA deficiency. To monitor metabolic disturbances in tissues of ADA-deficient mice, animals were sacrificed on postnatal day 15 and various tissues were rapidly collected and processed for nucleoside analysis. Consistent with observations made in ADA-deficient humans, there was a marked accumulation of adenosine (Fig. 5A) and 2'-deoxyadenosine (Fig. 5C) in the serum of ADA-deficient mice. Upon examination of various tissues, adenosine levels were found to be elevated in all tissues examined with the greatest accumulation occurring in the liver, kidney, and lung (Fig. 5B). 2'-Deoxyadenosine was also detected in all ADA-deficient tissues examined with the greatest accumulation occurring in the lymphoid organs (Fig. 5D). 2'-Deoxyadenosine was elevated more than 500-fold in the thymus to levels greater than 200 times that found in the serum of ADA-deficient mice (Fig. 5C). These studies demonstrate that there are severe metabolic disturbances in tissues of ADA-deficient mice.

ADA-deficient Mice Demonstrate Elevated Levels of dATP and Inhibition of S-Adenosylhomocysteine Hydrolase—The inhibition of AdoHcy hydrolase and the accumulation of dATP are common features monitored in red blood cells of ADA-

---

**Fig. 3.** ADA-deficient mice exhibit a severe lymphopenia and immunodeficiency. Panel A, lymphoid cell numbers from thymuses and spleens are given as mean cell numbers in millions ± S.E. (n = 7 for control organs [open bars] and n = 6 for ADA-deficient organs [solid bars]). Panel B, peripheral lymphoid cell counts are given as cells/μl blood ± S.E. (n = 29 for control values [open bar] and n = 16 for ADA-deficient values). Panel C, total immunoglobulin levels were measured in the serum of control (open bars) and ADA-deficient (solid bars) mice. Values are given as μg/ml ± S.E. (n = 7 for control mice; n = 6 for ADA-deficient mice). *** represents a significant difference from control values with p > 0.001.
deficient individuals (2, 16, 26). Disturbances in these pathways have been noted in mice treated with the ADA inhibitor 2'-deoxycoformycin (27); however, nothing is known with regard to the involvement of these toxic pathways in tissues of ADA-deficient individuals. To determine if these metabolic end points were effected in tissues of ADA-deficient mice, AdoHcy hydrolase enzymatic activity and dATP levels were monitored in various tissues (Fig. 6). In red blood cells, it was found that, as in humans, AdoHcy hydrolase activity was inhibited (Fig. 6A) and dATP accumulated to very high levels (Fig. 6C). AdoHcy hydrolase enzymatic activity was inhibited in all tissues examined with the greatest degree of inhibition seen in the thymus (85%) and spleen (90%) (Fig. 6B). dATP levels were increased in all tissues examined (Fig. 5D). The greatest accumulation of dATP was in red blood cells (Fig. 6C); however, among other tissues examined, the greatest accumulation (50-fold) was seen in the thymus (Fig. 6D). Therefore, ADA-deficient mice retain metabolic disturbances associated with ADA deficiency in humans. Moreover, of numerous tissues examined, the most severe metabolic disturbances occur in lymphoid tissues.

Lung, Bone, and Kidney Abnormalities Are Observed in ADA-deficient Mice—In addition to a combined immunodeficiency, ADA-deficient humans exhibit pathologic findings including, liver, kidney, adrenal, and bone abnormalities (28–30). To determine if there were pathological alterations in nonlymphoid tissues of ADA-deficient mice, tissues were harvested on postnatal day 15 and examined histologically. Severe histological observations were found in the lung of ADA-deficient mice (Fig. 7B). There was a large increase in the number of inflammatory cells in the lung, together with massive thickening and shedding of airway epithelium and occlusions of the airways with cellular debris and mucus (Fig. 7B). Consistent with these histological observations, mice began to show signs of tachypnea as early as postnatal day 12, and this labored breathing became increasingly severe up to the death of the animals between days 18 and 22. This severe pulmonary insufficiency is speculated to lead to the death of ADA-deficient mice, and the basis for the lung inflammation is under investigation. Bone

FIG. 4. Analysis of lymphoid cell distributions in ADA-deficient mice. Flow cytometry was performed on thymus (panel A) and spleen (panel B) cell preparations from aged-matched control and ADA-deficient mice. Values are given as mean percentages ± S.E. (n = 7 for control organs (open bars) and n = 6 for ADA-deficient organs (solid bars)). * represents a significant difference from control with p > 0.05; **, p < 0.01.

FIG. 5. Disturbances in adenosine and 2'-deoxyadenosine levels in tissues of ADA-deficient mice. Adenosine levels were quantitated in serum (panel A) or in various tissues (panel B). 2'-Deoxyadenosine in serum (panel C) and tissues (panel D) was resolved in the same profiles as adenosine. Mean values are given as nanomoles of adenosine or 2'-deoxyadenosine/mg of protein ± S.E. (n = 8 for control mice (open bars) and n = 4 for ADA-deficient mice (solid bars)). nd, not detected at a lower limit of 0.001 nmol/mg of protein.
Abnormalities of the rib cage were also noted in ADA-deficient mice (Fig. 7c). There was an enlargement of the costochondral junctions, as well as a severe rib curvature (Fig. 7d). Abnormal pathogenesis was also seen in the kidneys of ADA-deficient mice. Kidneys appeared normal in size but were dark red in comparison to control kidneys, which were pink (data not shown). Histological examination revealed a normal organization in the ADA-deficient kidney; however, there was a substantial increase in red blood cells found in glomeruli and convoluted tubules of ADA-deficient kidneys (Fig. 7f). These results suggest that ADA-deficient mice exhibit many of the nonlymphoid phenotypes observed in ADA-deficient humans.

**DISCUSSION**

ADA-deficient fetuses die perinatally in association with profound disturbances in purine metabolism and severe liver damage (8, 9). It has been shown recently that Ado expression in trophoblasts of the placenta is essential for prenatal survival in the mouse (18). In the current study, we used a transgenic approach to genetically restore ADA specifically to trophoblasts of otherwise ADA-deficient fetuses. Expression of Ado in trophoblasts was sufficient to rescue ADA-deficient fetuses from perinatal lethality, and, with loss of the placenta at birth, rescued mice were found to be completely ADA-deficient. These ADA-deficient mice provided for the first time the opportunity to examine the phenotypic and tissue-specific metabolic disturbances associated with ADA deficiency in postnatal mice.

Among the most striking observations made in ADA-deficient mice was a profound T and B cell lymphopenia. Furthermore, levels of serum immunoglobulins were greatly diminished, suggesting these animals suffer from a combined T and B cell deficiency. These features are consistent with the immune phenotype observed in ADA-deficient humans (2). The ability to analyze tissues from ADA-deficient mice, which are not easily attainable in ADA-deficient humans, allowed us to make additional observations regarding the immune phenotype associated with ADA deficiency. In the thymus of ADA-deficient mice, the percentage of CD4^+^CD8^−^ double-negative immature thymocytes increased, whereas the percentage of CD4^+^CD8^−^ double-positive thymocytes and CD4^+^ and CD8^+^ single-positive thymocytes decreased accordingly (Fig. 4A). This suggests that developing thymocytes are sensitive to the metabolic consequences of ADA deficiency at the transition from the double-negative to double-positive stage. This observation is consistent with those seen in mice treated with the ADA inhibitor 2′-deoxycoformycin (31, 32). However, the severe reduction in lymphoid cell number in the thymus suggests that there is a general lymphotoxicity associated with ADA deficiency, possibly a block at a prethymic stage of T cell development.

The ability to analyze individual tissues also provided novel
**Fig. 7.** Nonlymphoid phenotypes associated with ADA deficiency in mice. Lung tissues from control (panel a) and ADA-deficient mice (panel b) were collected at postnatal day 15, and sections were stained with hematoxylin and eosin. br, bronchile; a, alveoli; e, bronchiolar epithelium; *, immune cells in lumen of bronchile; large arrow, hypertrophied smooth muscle. Postnatal day 15 rib cages from control (panel c) and ADA-deficient (panel d) mice were stained with alizarin red and azure blue to monitor costochondral junctions. White arrows point to costochondral junctions. In panel d, denotes severe curvature of ribs seen in ADA-deficient mice. Hematoxylin/eosin-stained sections through lung (panel e) and spleen (panel f) kidneys. g, glomerulus; t, convoluted tubules; * and arrows in panel f denote areas of increased blood content in ADA-deficient kidneys. Scale bar in f = 100 μm for a, b, e, and f.

Information into potential metabolic mechanisms that lead to the combined immunodeficiency associated with ADA deficiency. There was a widespread accumulation of adenosine in all tissues examined in ADA-deficient mice, whereas 2'-deoxyadenosine accumulated predominantly in the thymus and spleen (Fig. 5). This suggests that 2'-deoxyadenosine cytotoxicity may be involved in the immunodeficiency observed. 2'-Deoxyadenosine can be cytotoxic to cells by directly inhibiting the enzyme AdoHcy hydrolase (15, 16). This inhibition can lead to the accumulation of AdoHcy that in turn functions as an inhibitor of transmethylation reactions that utilize S-adenosylmethionine as a methyl donor (2, 16). AdoHcy has also been shown to modulate Apo-1-mediated apoptosis (35). AdoHcy hydrolase enzymatic activity was inhibited in all tissues examined in ADA-deficient mice with the greatest degree of inhibition occurring in the thymus and spleen (Fig. 6A). Therefore, disruption of critical transmethylation reactions or induction of apoptosis following AdoHcy hydrolase inhibition may play a key role in the immune phenotype observed. Little is known regarding transmethylation reactions that may be affected; however, it is likely that ADA-deficient mice will serve as a tool to uncover potential transmethylation targets.

Accumulation of dATP in red blood cells of ADA-deficient patients has long been used as a benchmark for the severity of this metabolic disorder (2, 14, 34). Here, we show that dATP is not only elevated in red blood cells of ADA-deficient mice, but in all tissues examined (Fig. 6B). Levels of dATP in ADA-deficient thymuses and spleens were among the highest measured, suggesting that the phosphorylation of 2'-deoxyadenosine to dATP may play a role in the ensuing immune phenotype. Elevated dATP can interfere with deoxynucleotide metabolism by acting as an allosteric inhibitor of ribonucleotide reductase, an essential enzyme in the production of deoxynucleotides utilized in DNA synthesis and repair (13, 14, 35, 36). In the case of ADA deficiency, disruption of deoxynucleotide pools may influence lymphocyte proliferation, or their ability to repair DNA, and thus resulting in apoptosis. 2'-Deoxyadenosine-induced apoptosis in thymocytes has been shown to be dependent on expression of the DNA damage checkpoint protein p53 (32), suggesting that accumulation of DNA strand breaks may play a role in thymocyte apoptosis associated with ADA deficiency. More recently, it has been demonstrated that dATP is required for the activation of the caspase CPP32 that plays a pivotal role in the apoptotic pathway (37). In this manner, dATP accumulation in response to ADA deficiency may directly influence apoptosis in T and B lymphocytes. The existence of an ADA-deficient mouse will allow for further genetic manipulation and biochemical analysis to assess the role of these mechanisms in the immune phenotype associated with ADA deficiency.

Both AdoHcy hydrolase inhibition and dATP accumulation correlate with the high levels of 2'-deoxyadenosine seen in the thymus and spleen, suggesting 2'-deoxyadenosine cytotoxicity may be involved with the combined immunodeficiency seen. However, the accumulation of adenosine seen in the thymus and spleen suggests that perturbations in normal adenosine signaling may also play a role in the immune phenotype. Several studies suggest that adenosine signaling is involved in the normal development of the immune system (38–40), and adenosine and adenosine agonists have been shown to induce T cell death in the absence of ADA activity (11, 12). The expression of adenosine receptors in the developing immune system supports the hypothesis for the involvement of adenosine signaling (41, 42); however, functional analysis at the cellular and genetic levels are needed to assess the role of adenosine signaling in ADA-deficient immunodeficiency.

The fact that ADA-deficient murine fetuses die perinatally (8, 9), whereas ADA-deficient humans do not (2), suggests that there is a significant difference in the requirement for ADA during prenatal stages of murine and human development. This difference is manifested in the high levels of ADA that are found in the murine placenta (22) that do not appear to be
present in the human placenta (43). Despite this prenatal difference, results from this study show that once ADA-deficient fetuses have been rescued from perinatal lethality, the postnatal phenotypes observed are similar to those seen in ADA-deficient children. This similarity extends beyond the combined immunodeficiency to include a pulmonary insufficiency, bone abnormalities, and kidney findings. Many of these observations in humans were made during patient autopsy (29, 30), making it difficult to establish whether these phenotypes are a primary effect of ADA deficiency or secondary to the immunodeficiency. The metabolic disturbances seen in affected organs suggest that these phenotypes may be a primary consequence of ADA deficiency related to perturbations in adenosine signaling. Although the mechanisms by which the metabolic disturbances lead to tissue damage are still not understood, it is clear that these mice will provide a model system to learn more about pathways involved in the pathogenesis of these phenotypes in ADA-deficient mice and humans. In addition, these genetically engineered mice will make possible a wide range of biochemical and immunological experiments that are not permissible in humans, which will be directed at understanding the mechanism and treatment of ADA deficiency. In particular, ADA-deficient mice will aid the advancement of enzyme and gene therapy to ultimately treat this and related disorders in humans.

Acknowledgments—We thank Gregor Eichele and Stephen Elledge for the use of their microscopy and imaging equipment.

REFERENCES

1. Blaese, R. M. (1995) in The Metabolic and Molecular Basis of Inherited Disease (Scrivner, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) Vol. 3, pp. 3895–3909, McGraw-Hill, Inc., New York
2. Hershfield, M. S., and Mitchell, B. S. (1995) in The Metabolic and Molecular Basis of Inherited Disease (Scrivner, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) Vol. 1, pp. 1725–1768, McGraw-Hill, Inc., New York
3. Giblett, E. R., Anderson, A. J., Cohen, F., Pollara, B., and Meuwissen, H. J. (1972) Lancet 2, 1067–1069
4. Hershfield, M. S., Chaffee, S., and Sorensen, R. U. (1993) Pediatr. Res. 33, S35–S41
5. Bordignon, C, Notarangelo, L. D., Nobili, N., Ferrari, G., Casorati, G., Panina, P., Mazzolari, E., Maggioni, D., Rossi, C., Sercasi, P., Uguzo, A., G., and Meviglia, F. (1995) Science 270, 470–480
6. Biaese, R. M., Culver, K. W., Miller, A. D., Carter, C. S., Fleisher, T., Clerici, M., Shearer, G., Chang, L., Chiang, Y., Tolstoshev, P., Greenblatt, J. J., Rosenberg, S. A., Klein, H., Berger, M., Mullen, C. A., Ramsey, W. A., Muu, L., Morgan, R. A., and Anderson, W. F. (1995) Science 270, 475–480
7. Biaese, R. M. (1995) Pediatr. Res. 33, S49-S55
8. Wakamiya, M., Blackburn, M. R., Jurecic, R., McArthur, M. J., Gokse, R. S., Cartwright, J., Miti, K., Vaishnav, S., Belmont, J. W., Kellens, R. E., Finegold, M. J., and Caskey, C. T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3673–3677
9. Migghelsen, A. A. J., Breuer, M. L., van Roon, M. A., te Riele, H., Zarcher, C., Ossendorp, F., Toutain, S., Hershfield, M. S., Berns, A., and Valerio, D. (1995) Nat. Genet. 10, 279–287
10. Stiles, G. L. (1992) J. Biol. Chem. 267, 6451–6454
11. Szondy, Z. (1994) Biochem. J. 304, 877–885
12. Kizuki, H., Suzuki, K., Tadakuma, T., and Ishimura, Y. (1990) J. Biol. Chem. 265, 5280–5284
13. Ullman, B., Gudbj, L. J., Cohen A., and Martin, D. W., Jr. (1978) Cell 14, 365–373
14. Cohen, A., Hirschhorn, R., Horowitz, S. D., Rubinstein, A., Polmar, S. H., Hong., and Martin, D. W., Jr. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 472–476
15. Hershfield, M. S. (1979) J. Biol. Chem. 254, 22–25
16. Hershfield, M. S., Kredich, N. M., Ownby, D. R., Ownby, H., and Buckey, R. (1979) J. Clin. Invest. 63, 807–811
17. Shi, D., Winston, J. H., Blackburn, M. R., Datta, S. K., Hanten, G., and Kellens, R. E. (1997) J. Biol. Chem. 272, 2334–2341
18. Blackburn, M. R., Wakamiya, M., Caskey, C. T., and Kellens, R. E. (1995) J. Biol. Chem. 270, 23881–23894
19. Blackburn, M. R., Datta, S. K., Wakamiya, M., Vartabedian, B., and Kellens, R. E. (1996) J. Biol. Chem. 271, 15203–15210
20. Kruusen, T. B., Winters, R. S., Otey, S. K., Blackburn, M. R., Airhart, M. J., and Kellens, R. E. (1992) Teratology 43, 91–103
21. Gao, X., Blackburn, M. R., and Kruusen, T. B. (1994) Teratology 48, 1–12
22. Kruusen, T. B., Blackburn, M. R., Chrinsey, J. A., Mairht, M., and Kellens, R. E. (1991) Biol. Reprod. 44, 171–184
23. Malanchere, E., Huetz, F., and Coutinho A. (1997) Eur. J. Immunol. 27, 788–793
24. Morgan, G., Levinzky, R. J., Hugh, J. K., Fairbanks, L. D., Morris, G. S., and Simmonds, H. A. (1987) Clin. Exp. Immunol. 70, 491–499
25. Donofrio, J., Coleman, M. S., and Hutton, J. (1979) J. Clin. Invest. 62, 884–887
26. Kaminska, J. E., and Fox, I. H. (1980) J. Lab. Clin. Med. 96, 141–147
27. Ratech, H., Thorbecke, G. J., and Hirschhorn, R. (1981) Clin. Immunol. Immunopathol. 21, 119–127
28. Bollinger, M. E., Arredondo-Figa, F. S., Santuste, I., Schwartz, K., Hershfield, M. S., and Lederman, H. M. (1996) N. Engl. J. Med. 334, 1367–1371
29. Ratech, H., Greco, A., Gallo, G., Rimoin, D. L., Kamino, H., and Hirschhorn, R. (1985) Am. J. Pathol. 120, 157–169
30. Cederbaum, S. D., Kaitila, I., Rimoin, D. L., and Steinh, E. R. (1976) J. Pediatr. 89, 737–742