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Autoimmunity and Increased c-*myb* Transcription

Abstract. *A single recessive gene,* 1pr, *induces an autoimmune-lymphoproliferative syndrome in several strains of mice. The lymphoid organs of* 1pr/1pr *mice contained cells with increased amounts of* myb *RNA, which codes for a protein found in the nucleus. A similar human lymphoproliferative disorder also had an increase in c-*myb *expression. Mouse T cells induced by mitogens to proliferate did not express large amounts of* myb *RNA, indicating that marked* myb *expression is not a general feature of lymphocyte activation and proliferation.*

The generalized autoimmune diseases in mice and humans are a diverse group of disorders of unknown etiology characterized by excessive proliferation of T or B lymphocytes and production of autoantibodies (*1, 2*). In mice, a single recessive gene, *1pr*, induces autoantibody production and massive lymphocyte proliferation (*1*). Similarly, among the human autoimmune diseases, angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) is also characterized by autoantibody production and lymphocyte proliferation (*3, 4*). In our study of the molecular events responsible for these disease states, we examined the oncogene RNA products in involved lymphoid cells.

Oncogenes are the genetic elements in acute transforming retroviruses that are associated with the ability of these viruses to induce neoplastic transformation of cells (*5*). Cellular DNA sequences (c-*onc* genes) homologous with viral oncogenes (v-*onc* genes) have been conserved through evolution and can be found in most eukaryotic cells (*5–8*). Quantitative and qualitative alterations in different oncogene RNA's have been found in a large number of human and murine cancers (*9, 10*). We wondered whether a mechanism similar to this, but lacking a neoplastic transformation step, could play a role in the abnormal lymphocyte proliferation that characterizes most autoimmune diseases. To test this hypothesis we used oncogene probes to hybridize blots of electrophoretically separated polyadenylated [poly(A)$^+$] RNA from human peripheral blood mononuclear cells (PBMC's) and bone marrow or from spleens and lymph nodes from mice with and without autoimmune diseases. We found increases in the expression of the oncogene c-*myb* in both of these autoimmune diseases.

Three of the first oncogenes used as probes have been associated with lymphoid tumors: *abl, myc*, and *myb* (*9–11*). A fourth oncogene probe, *raf*, was studied because it has been mapped to chromosome 6 in the mouse, which contains the κ immunoglobulin light-chain gene (*12*) and has been associated with small cell lung carcinomas (*13*). Almost no difference in c-*abl* RNA expression was noted in a comparison of autoimmune and nonautoimmune mice (data not shown). The spleens of autoimmune NZB and BXSB mice, however, contained two to three times more c-*myc* RNA than those of nonautoimmune mice, and Southern blot hybridization showed no evidence of *myc* gene rearrangements or amplification (data not shown). The most striking and consistent finding was an elevation in the amount of c-*myb* RNA in tissues from subjects with a subset of autoimmune diseases, namely the spleens of MRL-*1pr/1pr* and C57BL/6-*1pr/1pr* mice and PBMC's and bone marrow cells of a patient with AILD.

The c-*myb* RNA's appeared as a major hybridizing band of 3.8 kilobases (kb) plus a fainter band of 4.2 kb (Fig. 1). The lowest amount of *myb* RNA was consis-

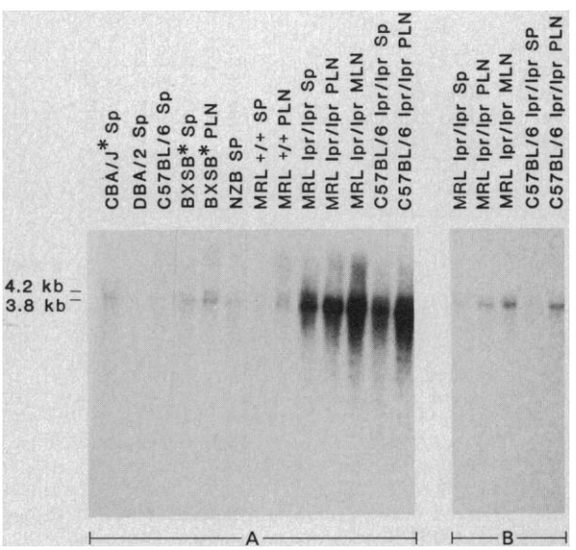

Fig. 1. Hybridization of v-*myb* probe to poly(A)$^+$ RNA (10 μg) from spleens (Sp), peripheral lymph nodes (PLN), and mesenteric lymph nodes (MLN) of mice (~6 months old) of indicated strain. The guanidine thiocyanate method (*22*) was used to rupture cells and to denature ribonuclease simultaneously. The RNA was separated from DNA and proteins by centrifugation through cesium chloride (*23*). For final purification, the RNA was extracted once in chloroform-phenol and once in chloroform. The poly(A)$^+$ RNA fraction was obtained by passing the total purified RNA twice over an oligo(dT)-cellulose column (*24*). Poly(A)$^+$ RNA (10 μg) was denatured in 14 m*M* methylmercury hydroxide and subjected to electrophoresis in 1.5 percent agarose gels containing 5 m*M* methylmercury hydroxide. The RNA was blotted onto *O*-diazophenyl thioether paper and prehybridized and hybridized with a v-*myb* DNA probe that had been labeled with ^{32}P by nick translation to a specific activity of 1.4×10^8 to 2.0×10^8 count/min per microgram (*9*). The films were scanned with a CS300 scanning densitometer (Hoefer Scientific). In some cases, after photography of the autoradiographs certain lanes were cut out and juxtaposed with others from the same gel to illustrate more clearly important comparisons. The v-*myb* probe was a 1.3-kb Kpn I–Xba I fragment isolated from cloned avian myeloblastosis virus (*11*) and subcloned in pBR322. (A) Autoradiographs of the v-*myb* probe hybridized to blots of spleen and lymph node poly(A)$^+$ RNA after a 12-hour exposure. (B) The right-most six lanes of (A) after a 1-hour exposure. All mice were female except those designated by an asterisk (*).

tently present in spleens of nonautoimmune mouse strains such as CBA/J, DBA/2, and C57BL/6 (the CBA/J control was used to define the normal amount of *myb* RNA). An intermediate amount (about twice normal as determined by densitometric scanning) was found in spleens of female NZB and male BXSB mice, which develop a form of autoimmunity characterized by B cell hyperactivity and moderate lymphadenopathy later in the course of the disease (*2*). A similar amount of *myb* was present in spleens from MRL+/+ mice, which develop mild autoimmunity late in life (*1*). Although the lymph nodes were only slightly enlarged in BXSB male and MRL+/+ mice, the amount of *myb* RNA was noticeably higher (about five times normal) in poly(A)$^+$ RNA from these lymph nodes as compared to equal amounts of RNA from the spleen. The highest amounts of c-*myb* RNA were present in lymphatic organs of C57BL/6-*lpr*/*lpr* and MRL-*lpr*/*lpr* mice, which characteristically develop massive lymphadenopathy and produce autoantibodies to DNA, immunoglobulin, and other self antigens (*2*). The spleens of these animals contained large amounts of *myb*-hybridizing RNA (about 16 times normal), and even more *myb* RNA was found in the enlarged peripheral lymph nodes (32 times normal) and mesenteric lymph nodes (56 times normal) of these mouse strains. The *lpr* gene is implicated in these changes because there was no such greatly increased *myb* RNA in spleens or nodes of C57BL/6+/+ and MRL+/+ strains, which are coisogenic with the strains having large amounts of *myb* RNA (except the *lpr* gene). The *lpr* gene seems to be required in homozygous form to result in high *myb* expression because spleens from (C57BL/6-*lpr*/*lpr* by MRL+/+)F_1 mice did not express high *myb* RNA levels (data not shown) (*14*).

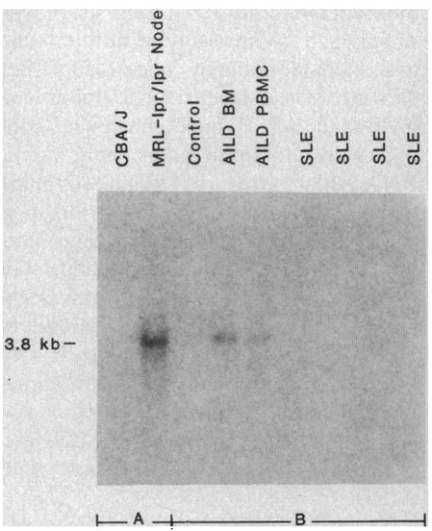

Fig. 2. Hybridization of v-*myb* probe to poly(A)$^+$ RNA (10 μg) from peripheral blood mononuclear cells (PBMC) from a normal human subject, patients with systemic lupus erythematosus (SLE) or AILD, or bone marrow (BM) cells from the AILD patient. For comparison, 10 μg of poly(A)$^+$ RNA from spleen cells of male CBA/J mice cells or lymph node cells of MRL-*lpr*/*lpr* mice was applied to the same gel (A). Isolation, blotting, and hybridization of RNA was carried out as described in the legend to Fig. 1. Large amounts of *myb* RNA were found in bone marrow cells, and a smaller amount was found in PBMC of a patient with AILD. No detectable *myb* RNA was found in equal amounts of poly(A)$^+$ RNA from PBMC from other patients with systemic lupus erythematosus or from the normal control (B).

A similar experiment was performed to compare the *myb* RNA levels in PBMC's from normal human controls and patients with two different autoimmune diseases (Fig. 2). An increased amount of c-*myb* RNA was present in PBMC's from only one of the patients, the one with AILD. This disease is characterized by massive lymphadenopathy, hepatosplenomegaly, polyclonal gammopathy, and autoantibody production (*4*, *15*). This patient's bone marrow cells contained even greater amounts of *myb* RNA than did the PBMC's. The amounts of *myb* RNA in patients with systemic lupus erythematosus were not appreciably higher than those in PBMC's of normal human controls. The AILD RNA preparations did not show consistent changes in amounts of *abl* or *myc* RNA's (data not shown). This indicates that the high *myb* level is a specific alteration of transcription of one but not all oncogenes and that there is no generalized increased transcription of genes in the AILD patient.

In experiments to determine whether there was a correlation between c-*myb* RNA levels and proliferation of normal lymphocytes, B and T cell–specific mitogens were used to induce cell division in normal spleen cells. Spleens from 50 female DBA/2 mice were cultured for 4 and 18 hours with either concanavalin A (Con A; 5 μg/ml) or lipopolysaccharide (LPS; 50 μg/ml) (*16*). Poly(A)$^+$ RNA from equal numbers of cells (3×10^8) was prepared, subjected to electrophoresis, blotted, and hybridized with a probe for c-*myc*, v-*myb*, or v-*raf* (Fig. 3). At 4 hours *myc* expression was increased in the LPS-stimulated cultures, and at 18 hours *raf* expression was slightly increased in both LPS- and Con A–stimulated cultures. *Myb* RNA expression was minimal in nonstimulated spleen cells and was not increased at either time. Thus, high *myb* expression does not reflect nonspecific stimulation of proliferation but appears to be related to certain cells at particular stages of differentiation.

The mouse *lpr* gene is responsible for accumulation of abnormal T cells in the spleen. An even greater concentration of these cells is found in the enlarged peripheral and mesenteric lymph nodes (*17*). This is consistent with our results (Fig. 1) showing that increased *myb* expression is associated with the abnor-

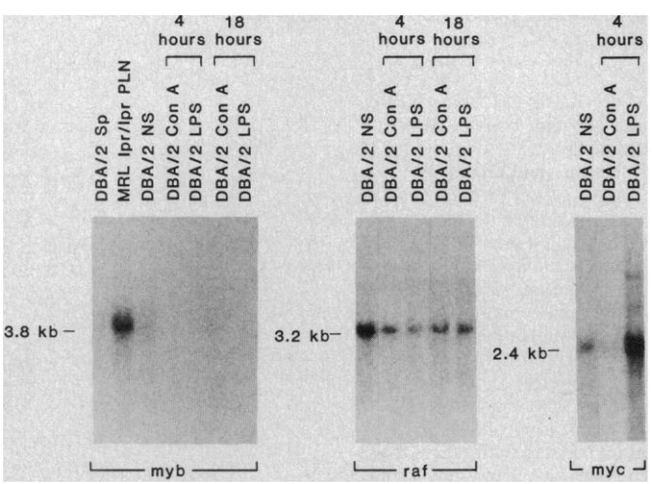

Fig. 3. Equal numbers of spleen (Sp) cells from female DBA/2 mice were stimulated for 4 and 18 hours with Con A (5 μg/ml) or LPS (50 μg/ml) under standard culture conditions. The poly(A)$^+$ RNA from 3×10^8 cells (~15 μg) was subjected to electrophoresis, blotted, and hybridized with oncogene probes [v-*myb*, as in Fig. 1; v-*raf*, the 0.67-kb Xho I–Sst II fragment isolated from cloned murine sarcoma virus 3611 (*12*); and c-*myc*, a 5.5-kb Bam HI fragment isolated from a mouse plasmacytoma S107 bacteriophage library (*9*)]. Large amounts of *myc* RNA were present at 4 hours in the LPS-stimulated cultures, but little *raf* RNA and no *myb* RNA was detected at 4 and 18 hours. The *raf* but not *myb* RNA was observed in the Con A–stimulated cultures at 18 hours. The amount of *myb* RNA in nonstimulated (NS) cells was measured at time zero.

mal T cells that pack the lymph nodes of *lpr/lpr* mice (*17*). It is also consistent with observations that the amount of *myb* RNA is high in immature, actively dividing hemopoietic cells of the chicken (*18*) and in thymus and immature, incompletely differentiated lymphocytic cells and tumors in mice (*19*).

Our results confirm the previous observation that the amount of *myb* RNA is substantial in the thymus (*18*), an organ with large numbers of proliferating T cells. However, high *myb* expression does not appear to be a feature of all cells that are undergoing proliferation because, of the lymph node cells from *lpr/lpr* mice that contained large amounts of *myb* RNA, only 6 percent were proliferating (*20*); also, mitogen-stimulated spleen cells did not express high *myb* even though they did express *myc* and *raf*. It is possible that *myb* is only expressed for a limited period during mitogen-induced proliferation and that, during the times chosen for study, transient *myb* expression was not detected. However, if that were the case, the high *myb* expression in *lpr/lpr* lymph nodes and in AILD would still indicate that a large number of cells is arrested in a particular stage of the cell cycle. Taken together, the data suggest that *myb* expression is high in cells for limited periods during differentiation rather than only during proliferation.

The results of other studies have implicated abnormal lymphoid differentiation as a common feature in autoimmune diseases (*1, 2, 21*). Individual accelerating genes, such as *lpr*, probably induce autoimmune phenomena by causing differential expression of certain genes (*2, 17*). As a step toward understanding the details of pathogenic gene expression, our results are apparently the first definitive association of increased oncogene expression with murine and human autoimmune disease. We suggest that the high levels of *myb* RNA in lymph nodes of *lpr/lpr* mice, known to be packed with the abnormal T cells associated with this autoimmune disease, may help identify the cells that act in the disease process and may provide one way to explore the genetic basis of autoimmune disease states.

JOHN D. MOUNTZ*
ALFRED D. STEINBERG
DENNIS M. KLINMAN
HOWARD R. SMITH
National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, Maryland 20205
J. FREDERIC MUSHINSKI
Laboratory of Genetics, National Cancer Institute, Bethesda, 20205

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* To whom correspondence should be addressed at the Cellular Immunology Section, ARB:NIADDK, National Institutes of Health, Building 10, Room 9N-218, Bethesda, Md. 20205.

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Prolonged Ca^{2+}-Dependent Afterhyperpolarizations in Hippocampal Neurons of Aged Rats

Abstract. *The possibility that calcium is elevated in brain neurons during aging was examined by quantifying afterhyperpolarizations induced by spike bursts in CA1 neurons of hippocampal slices from young and aged rats. The afterhyperpolarizations result from Ca^{2+}-dependent K^+ conductance increases and are blocked in medium low in Ca^{2+} and prolonged in medium high in Ca^{2+}. The afterhyperpolarization and associated conductance increases were considerably prolonged in cells from aged rats, although inhibitory postsynaptic potentials did not differ with age. Since elevated intracellular Ca^{2+} can exert deleterious effects on neurons, the data suggest that altered Ca^{2+} homeostasis may play a significant role in normal brain aging.*

Although it is well established that a variety of structural, chemical, and physiological changes occur in the mammalian brain during normal and pathological aging (*1*), little is known about the mechanisms that induce these age-dependent alterations. Among the few consistent microelectrophysiological changes observed in rats during aging are an impaired capacity of hippocampal synapses to exhibit frequency or long-term potentiation in response to repetitive stimulation (*2*) and altered patterns of transmitter release at the neuromuscular junction during stimulation (*3*). However, some nonsynaptic neurophysiological changes are also seen with aging (*4*).

Elevation of the extracellular Mg^{2+}:Ca^{2+} ratio selectively ameliorates the age-related deficit in hippocampal frequency potentiation, both in vitro (*5*) and in vivo (*6*). High Ca^{2+} impairs frequency potentiation in various preparations by saturation of binding sites for release or by rapid transmitter depletion (*7*); since Mg^{2+} competitively inhibits these Ca^{2+} actions (*7*), the beneficial effects of Mg^{2+} on hippocampal synaptic potentiation in aged rats have suggested that Ca^{2+} may be functionally elevated in aged hippocampal cells (*5, 6*). Other results, including changes in Ca^{2+} exchange or uptake in aged rat synaptosomal preparations (*8*), are at least consistent with the possibility that brain Ca^{2+} homeostasis is altered with age. If elevated brain Ca^{2+} does occur, the implications for both physiological and structural mechanisms of brain decline during aging might be substantial. That is, neurophysiological functions are likely to be affected by high Ca^{2+}, and a rise in intracellular Ca^{2+} seems to accelerate the structural disintegration of nonneural or neural cells that follows cellular trauma (*9*). These and other data have led to a growing interest in the hypothesis that an elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) could be one important factor in the complex set of processes that pre-