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LUPUS-RELATED TRANSCRIPT HOMOLOGOUS TO ABELSON-MURINE LEUKEMIA VIRUS

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SUMMARY: In the present study, we found the expression of transcripts homologous to Abelson murnie leukemia virus (A-MuLV) in lupus-prone mice and nonautoimmune mice. One of them, as the lupus-related transcript, is expressed in only lupus-prone mice and induced by bacterial lipopolysaccharide (LSP). We could not induce the transcript by LSP in nonautoimmune mice that we examined. The significance of the transcript autoimmune events in lupus-prone mice is discussed.

In systemic autoimmune diseases such as systemic lupus erythematosus(SLE), connecitve tissue inflammation and microvascular damage are the most prominent pathologic changes, but most of the fundamental causes of autoimmune disorders are still unknown. Initial studies of murine models of SLE have provided evidence for immune complexes containing the major envelope glycoprotein, gp70, of endogenous retroviruses and the corresponding antibody(1). It is clear that mice inherit determinants for their endogenous retroviruses as chromosomal genes(2). Certain viral gene segments seem to be expressed selectively depending on their site of integration into chromosomal loci, in which induction is linked to the differentiated state of the cells(3).

Abelson murine leukemia virus(A-MuLV) is a replication-defective retrovirus and induces B cell lymphomas in vivo and is able to transform both lymphoid and fibroblastic cells in vitro(4). C-myc RNA expression markedly increases in autoimmune mice(5), which may interact with various events in B and/or T cell activation. In the present study, we studied the expression of transcripts homologous to A-MuLV in lupus-prone mice and

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nonautoimmune mice using cloned probes specific the A-MuLV genome, and the induction by bacterial lipopolysaccharide(LPS). The significance of these transcripts in relation to autoimmune events and c-myc oncogene expression in lupus-prone mice is discussed.

MATERIALS AND METHODS

Mice: Mice used in this study were 2 day-16 weeks old. BXSB male for lupus-prone mice, BXSB female, C57BL/6 and BALB/c mice for control mice were maintained in our laboratory.

RNA isolation and northern blot hybridization: Total RNA from spleen cells was prepared by the guanidinium-thiocyanate-cesium chloride procedure, denatured in glyoxal, and electrophoresed in 1.4% agarose gels(20 μg per sample)(6). RNA was transferred to nylon membranes(Biodyne, Pall Inc. New York). The filters were hybridized(7) in a solution including 50% formamide, 0.75M sodium chloride, and 10% dextran sulfate at 42 °C with nick-translated ^{32}P subcloned DNA probes from A-MuLV genome, which was a gift from JCRB(8) and v-myc DNA(Takara Chemical Inc.)(specific activity, 1.5×10^8 cpm per mg). The final washes were done under conditions with 0.3xSSC(15 mM sodium chloride and 1.5 mM sodium citrate, pH7.2) at 45 °C for 1 hr each, before autoradiography.

Lipopolysaccharide(LPS) stimulation: The LPS preparation(Lipopolysaccharide E. Coli 055, Difco Inc.) was diluted to the desired concentrations with sterile saline and used at a final volume of 0.4ml to inject the mice intraperitoneally(i.p.).

RESULTS AND DISCUSSION

Expression of the transcripts homologous to A-MuLV

We first detected the 6.5 and 5 kb transcripts homologous to A-MuLV in both lupus-prone mice and control mice, and the 8 kb transcript homologous to A-MuLV in only lupus-prone mice(Fig.1), which is a same size as neither wild type transcript of A-MuLV(v-abl) or cellular homolog transcript of A-MuLV(c-abl).

Induction of the lupus-related transcript with LPS stimulation

Recent data demonstrates that bacterial LPS activates xenotropic virus in cultured spleen cells from various strains of mice(9). We next investigated the in vivo effect of LPS on the expression of the lupus-related transcript homologous to A-MuLV genome in relation to the activation of xenotropic virus. In fig.2, we showed the induction of the transcript with LPS injection in only BXSB male for the lupus-prone mice, but not in BXSB female for normal control mice. First, 2-month-old BXSB male mice with

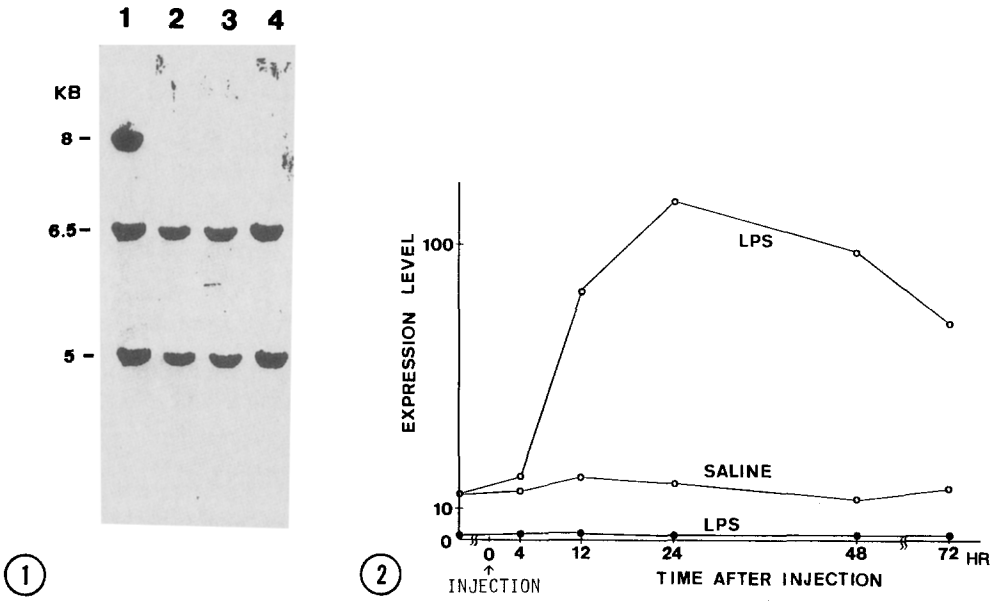

Fig.1: Expression of the transcripts homologous to A-MuLV genome in mice. Lane 1: BXSB male; 2, BXSB female; 3, C57BL/6; 4, BALB/c. Each mice strains were 2-month-old.

Fig.2: Response of the expression of lupus-related transcript homologous to A-MuLV genome after a single i.p. injection of 25 μg of LPS and saline in 2-month-old BXSB mice. Open circle: BXSB male mice; closed circle, BXSB female mice. Each point represents the mean value for three mice.

relatively high expression of lupus-related transcript homologous to A-MuLV, BXSB female and other control mice without its expression were injected i.p. with 25 ug of LPS. Compared with preinjection levels, amounts of expression of the transcript increased significantly 4 hr after the injection in both mice(fig.2). The amounts peaked between 12 and 24 hr and returned to the pretreatment levels within 72 hr. At the peak response, the expression level were about 10 times higher than those before injection of LPS.

At last we examined the relation to the expression of the transcript and disease signs, such as proteinuria, lymphoadenopathy and so on(10). Mountz et al and we reported the relation to c-myc oncogene expression and disease signs in lupus-prone mice and SLE patients(5, 11). In table I, we showed the similar expression pattern of the transcript as c-myc oncogene expression during the disease process and the transcript expression level could be related to the disease activity in lupus-prone mice.

Table I : Correlation of lupus-related transcript, c-myc transcript and lupus manifestations

age	lupus-related transcipt(8kb)	c-myc transcript	disease*
1 day	+	+	-
1 month	+	+	+
3 month	+++	+++	+++
6 month	+++	+++	+++

* Disease manifestations are glomerulonephritis, moderate lymphoproliferation, degenerative coronary artery disease, and some kinds of autoantibodies.

A-MuLV transforming functions are mediated by 120 kd protein(p120). This protein is a hybrid molecule containing Moloney-murine leukemia virus(M-MuLV) gag gene structural proteins p15, p12, and a small part of p30, as well as a MuLV-unrelated component encoded by abl sequence(12). Many of naturally occurring A-MuLV variants have also been isolated(13). Variants that express proteins as small as 90 kd was shown to retain transforming activity for fibroblasts(14). A number of transforming retroviruses including A-MuLV have been shown to possess closely associated kinase activity with specifiity for tyrosine phosphorylation(15). It is possible that the phosphorylation change by lupus-related transcript homologous to A-MuLV genome could effect on unknown gene activation which is important for pathogenesis of lupus. We further study the cloning and characterization of the lupus-related transcript which might have key role for pathogenesis of the disease.

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