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EXPRESSION OF VIRAL ANTIGENS ON THE MEMBRANE OF NORMAL AND LEUKEMIC THYMOCYTES OF AKR MICE

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Expression of antigenic determinants of structural proteins G-MuLV (p10, p12, p30, gp14, gp17) and R-MuLV (gp69/71, p15) on thymocytes of normal and leukemic AKR mice was stuided by the membrane immunofluorescence method. A sharp difference was found with respect to this feature between normal and malignant thymocytes. The possible role of antigens of structural viral proteins MuLV, expressed on the membrane of leukemic cells, in antitumor immunity is discussed.

KEY WORDS: immunofluorescence; antigenic determinants; expression; structural proteins MuLV; thymocytes.

Mouse leukemia cells induced by oncogenic viruses of the C type contain antigenic determinants of viral structural proteins on their membrane [2, 3, 12, 13, 15]. Some of them are responsible for the reaction of the cells with hyperimmune mouse sera [6, 11, 13], and antibodies against structural proteins MuLV have been found in the sera of normal mice [8, 11]. It has recently been shown that antigenic determinants of internal structural proteins MuLV are components of glycolyzed polyprotein molecules which are themselves integral components of the cell membrane [11, 13, 15]. They may perhaps partly determine the tumor phenotype and they are evidently largely responsible for immunologic relations between the host and the tumor.

In the investigation described below an immunofluoresence method was used to study expression of antigens of viral structural proteins on the membrane of normal and leukemic thymocytes from AKR mice.

METHODS

AKR mice were obtained from N. N. Medvedev (Hybrid Mice Nursery, N. F. Gamaleya Institute of Epidemiology and Microbiology). Thymocytes from AKR mice aged 2-4 and 8 months served as the test cells. Goat sera against individual structural proteins, products of the env gene (gp14 and gp17 G-MuLV, gp69/71 R-MuLV) and products of the gag gene (p10, p12, p30 G-MuLV and p15 R-MuLV) were obtained by J. T. August and M. Strand USA) [14]. The sera were absorbed by sheep's red blood cells and spleen cells of normal BALB/c mice and were neutralized with mouse γ-globulin as described previously [2]. The immunofluorescence test was carried out on an artificial monolayer of living cells obtained by incubation of a suspension for 10 min on a slide in a humid chamber. Rabbit antibodies against sheep γ-globulin were obtained by B. P. Bogovskii on a γ-globulin glutarate immunosorbent [4] and were conjugated with fluor-

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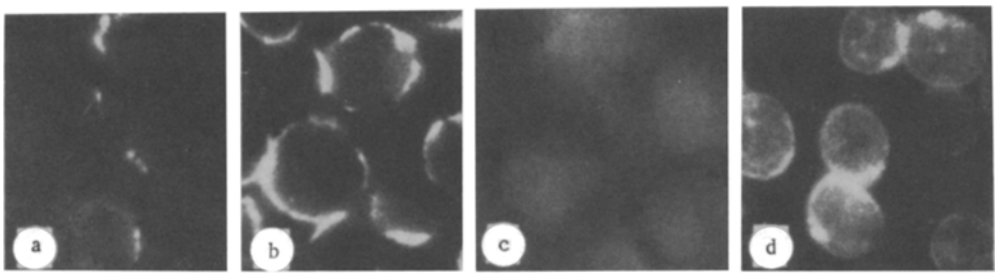

Fig. 1. Membrane fluorescence: a) normal thymocytes of AKR mouse aged 2 months, treated with anti-gp69/71 serum; b) leukemic thymocytes of AKR mouse aged 8 months, treated with anti-gp69/71 serum; c) living Rauscher erythroblastosis cells treated with anti-gp17 serum; d) Rauscher erythroblastosis cells treated with anti-gp17 serum after fixation with acetone.

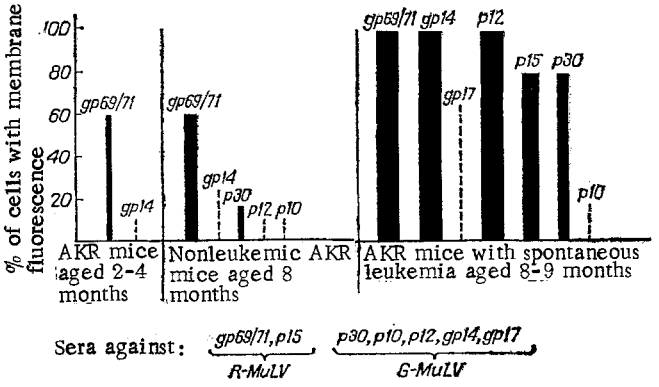

Fig. 2. Dynamics of expression of antigenic determinants of MuLV proteins on normal and leukemic thymocytes of AKR mice. Height of column reflects greatest percentage of cells with membrane fluorescence for each test serum; width of column conventionally reflects intensity of reaction. I) AKR mice aged 2-4 months, II) nonleukemic AKR mice aged 8 months, III) AKR mice aged 8-9 months with spontaneous leukemia.

escein. Labeled antibodies were absorbed with liver powder and sheep's red blood cells and neutralized with mouse γ-globulin. Full details of the method of absorption of the sera and conduct of the immunofluorescence test were given previously [2].

RESULTS

The thymus, spleen, and lymph nodes of mice aged 2-4 months were not enlarged and 40-60% of the thymocytes reacted with antiserum against glycoprotein gp69/71. The intensity of membrane fluorescence in these experiments was low and was represented conventionally as + (Fig. 1a). Trace relations with anti-gp14 serum were found in 8-12% of cells. Antigenic determinants of proteins p10, p12, gp17, and p30 were not found. Consequently, only antigenic determinants gp69/71 were clearly distinguished on the thymocytes of young mice by the immunofluorescence method.

In mice aged 8 months, with no marked anatomical features of leukemia, membrane fluorescence with antisera against gp69/71 also was found in 60% of thymocytes, but the intensity of the reactions was greater (+++) than in young mice. Trace reactions with serum against gp14 were given by 13-25% of cells. Weak membrane fluorescence with sera against structural proteins which are products of the gag gene was observed in a very small percentage of cells. The reaction of clearest intensity (+) was observed with serum against p30 (15-17% of cells) and trace reactions were given by sera against p10 (5-11%) and p12 (5-10%). In old mice

without leukemia more than half of the thymocytes thus had considerable quantities of antigenic determinants for gp60/71 on their membrane and only 15-17% of these cells had antigenic determinants for p30.

In 8-month AKR mice with spontaneous leukemia the thymus, spleen, and axiliary, brachial, and mesenteric lymph nodes were considerably enlarged. The thymocytes of these mice gave very strong membrane fluorescence (++++) with sera against gp69/71 (Fig. 1b), gp14, and p12. Each of these sera reacted with 90-100% of cells. Antiserum against p15 R-MuLV gave a rather weaker reaction (+++; 80% of cells). A moderate reaction (++) was observed with antisera against p30 (70-80% of cells). Antiserum against gp17 in one of three experiments did not react at all and in two experiments gave trace reactions (35 and 65% of cells respectively). Trace reactions also were observed (5-16% of cells) with antisera against p10.

Membrane fluorescence on intact leukemic cells of AKR mice thus showed strong expression of antigenic determinants of proteins that are products of the env gene: gp69/71 and gp14. Glycoprotein gp69/71 was expressed on leukemic thymocytes more strongly and on a larger number of cells than on thymocytes of mice of the same age without leukemia. On virtually all the thymocytes of mice with leukemia antigenic determinants for gp14 were strongly expressed, whereas on cells of nonleukemic mice antiserum against gp14 gave trace reactions with only 13-25% of cells.

Thymocytes of AKR mice with leukemia differed from preleukemic thymocytes also by the strong expression of antigenic determinants for p12 G-MuLV, which were revealed both by serum against p12 and also, evidently, by serum against p15 R-MuLV, which cross reacts with it [14]. The dynamics of expression of antigenic determinants of structural proteins on the thymocyte membrane is shown schematically in Fig. 2.

Expression of gp69/71 and gp14 on leukemic thymocytes appeared quite comparable on subjective evaluation of the membrane fluorescence reaction: Both antisera reacted with 90-100% of cells with an intensity of ++++, whereas gp14 was practically never found on normal thymocytes, and gp69/71 was expressed in quantities clearly detected by the immunofluorescence method (Fig. 2). This observation contradicts the coordinated synthesis of proteins which are products of one gene, and it can be explained either by inaccessibility of antigenic determinants of gp14 of normal thymocytes for antibodies, or by its stronger expression of lymphoma cells because of the functioning of the recently described MCF recombinant genome in them [7, 9].

An unexpected finding was the absence of expression of gp17 on intact cells — both normal and leukemic. Antigenic determinants of gp17 may perhaps have been screened by certain membrane structures and were consequently inaccessible for antibodies. This suggestion is supported by results obtained by the writers with Rauscher erythroblastosis cells. Anti-gp17 serum did not react with intact cells, whereas cells fixed with acetone gave strong membrane fluorescence (Fig. 1d). Fixation evidently led to conformational changes in the membrane, revealing determinants of gp17.

Sera against p30, p12, and p15 in the presence experiments evidently revealed antigenic determinants of internal structural proteins which are components of glycolyzed polyprotein molecules expressed on the membrane of tumor cells [13, 15]. Serum against p15 R-MuLV possibly cross-reacted with that part of the amino acid sequences of the polyprotein molecule which corresponds to structural protein p12 G-MuLV, responsible for the subgroup specificity of leukemias of the G-type [14].

Unlike other workers [11, 15], we did not obtain significant results in experiments with anti-p10 serum. These differences may perhaps be explained by differences in the sensitivity of the methods used.

The biological importance of the presence of antigenic determinants of MuLV proteins (products of the gag gene) in the composition of glycolyzed polyproteins on the tumor cell membrane is not known [11, 13, 15]. The cause of expression of polyprotein precursors of internal structural MuLV proteins on the cells, according to some workers [7, 9], is linked with the functioning of the recombinant viral genome.

Expression of different antigenic determinants of virus proteins on the tumor cell, in our opinion, may play a role in immunity, for antibodies against gp70, p30, and p15 have been found in homologous animals giving an immunologic response to type-specific determinants [1, 11].

It has recently been shown that gp70 of recombinant genomes are identical with one another and differ from the p70 of the hypothetical parent viruses [5]. These differences may perhaps include changes in certain type determinants, and indeed the type determinants of recombinant gp70 may perhaps be true leukemia-specific antigens. In this context it is interesting to study peptide maps of gp14 which, like gp69/71, is a product of the env gene, expressed in large quantities on the membrane of leukemic cells of AKR mice.

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