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Detection of Antigen-(AKR MuLV gp70)-Specific Circulating Immune Complexes (CIC) in Mice with Lymphomas *

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A. Introduction

Circulating immune complexes (CIC) probably play an important role in a variety of human and animal neoplastic diseases. CIC may represent the major part of the humoral factors which inhibit cell-mediated reactivity to tumor cells [1]. Furthermore, CIC in tumors seem to be a prognostic factor for the course of the disease [2, 3]. In some reports leukemias and lymphomas in man have been reported to be associated with immune complexes [7, 8, 10, 11] and have been shown to represent an unfavorable prognostic factor [2].

The specificity of the antibody IgG moiety and hence the nature of the complexed antigens in CIC in leukemias and lymphomas is unknown. Recently we showed the presence of CIC with retroviral antigens in some patients with CML blast crisis [5]. Retroviruses as possible causative factors of leukemias and lymphomas in men and animals are therefore probably involved in the formation of CIC. To resolve this question, mice with lymphomas of different origin were examined as model systems for the presence of CIC and especially for the presence of CIC containing retroviral envelope proteins.

B. Methods

CIC with undefined antigen were detected by a C1q-binding test according to Wehler et al. [12], using the ELISA technique. CICs with AKR MuLV gp70 were determined by the ELISA technique using a AKR MuLV gp85 rabbit antibody (gift of Dr. G. Hunsmann, Institut für Immunbiologie, Freiburg) as coating serum.

The serum samples were diluted 1 : 100 in assay buffer (PBS, 0.1% Tween 20, 0.5% Trasylol, 0.1 m*M* Thimerosal, 1% BSA), added to the well, and incubated at 4 °C overnight. AKR MuLV gp70 containing immune complexes present in the serum were determined by adding peroxidase-coupled rabbit anti-mouse IgG diluted in assay buffer. *O*-phenylenediamine-2HCl was used as substrate. The absorbance was measured at 450 nm with a Titertek Multiscan or a SEI/Kontron SLT 210. AKR MuLV gp70 and antibodies against AKR MuLV gp70 were detected with the ELISA technique [4, 9]. All mouse strains were from the breeding colony of the Institute of Biology of the GSF.

C. Results

Three different types of murine lymphomas have been examined for the presence of CIC, the spontaneous T-cell lymphomas of AKR mice, the X-ray induced T-cell lymphomas of C57Bl/6 mice, and the spontaneous late B-type lymphomas of low leukemia mouse strains (BALB/c, X/Gf, CBA). Age-matched healthy animals served as controls (AKR: 5–8 months; C57Bl/6: 5–8 months; BALB/c, NMRI, X/Gf, CBA: older than 18 months).

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I. Spontaneous AKR T-Cell Lymphoma

The spontaneous T-cell lymphomas of AKR mice have been found to develop during months 7–10. AKR mice with lymphomas (with the exception of two animals) did not show elevated CIC or AKR MuLV gp70 containing CIC in comparison to age-matched AKR mice or control mice of other strains (Figs. 1, 2). The AKR mice harboured a remarkable amount of AKR MuLV gp70 of 6–14μg/ml serum

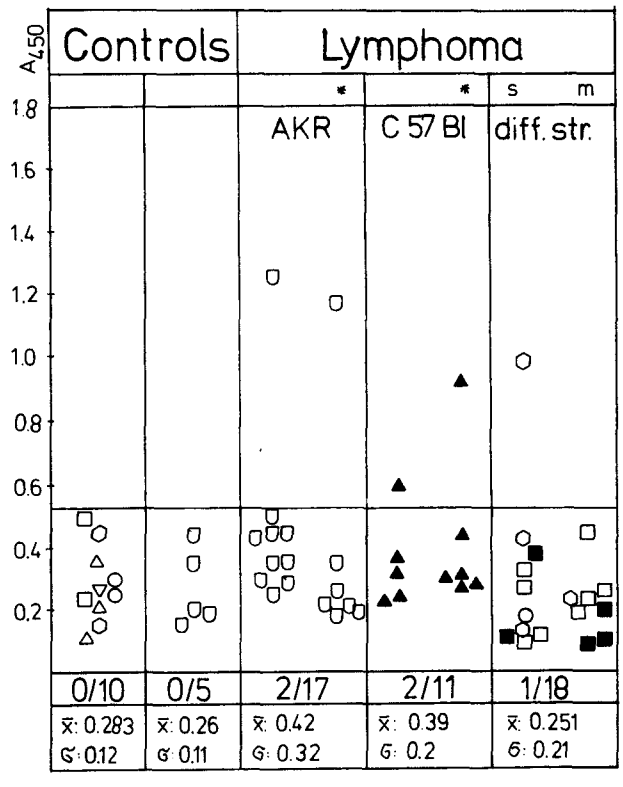

Fig. 1. C1q-binding circulating immune complexes in the sera of lymphoma-bearing mice. *Symbols:* ⌓ AKR, □ BALB/c, ◇ X/Gf, ○ CBA, △ C57Bl/6, ▽ NMRI. *Closed symbols* represent animals which had been irradiated with X-rays (column 4) or ^{227}Th (column 5). * The animals in these columns had been pretreated with alkylphospholipid (ALP); *s*, animals with lymphoma only; *m*, animals with an additional tumor to the lymphoma; the *horizontal lines* represent the mean value plus 2σ of the respective control animals; *column 1*, healthy animals of different mouse strains; *column 2*, healthy AKR mice; *column 3*, AKR mice with lymphoma; *column 4*, C57Bl/6 mice with lymphoma; *column 5*, mice of different strains with lymphoma. At the *bottom of the columns* the number of positive mice to the number of mice examined is indicated together with the mean value of the group plus standard deviation (σ)

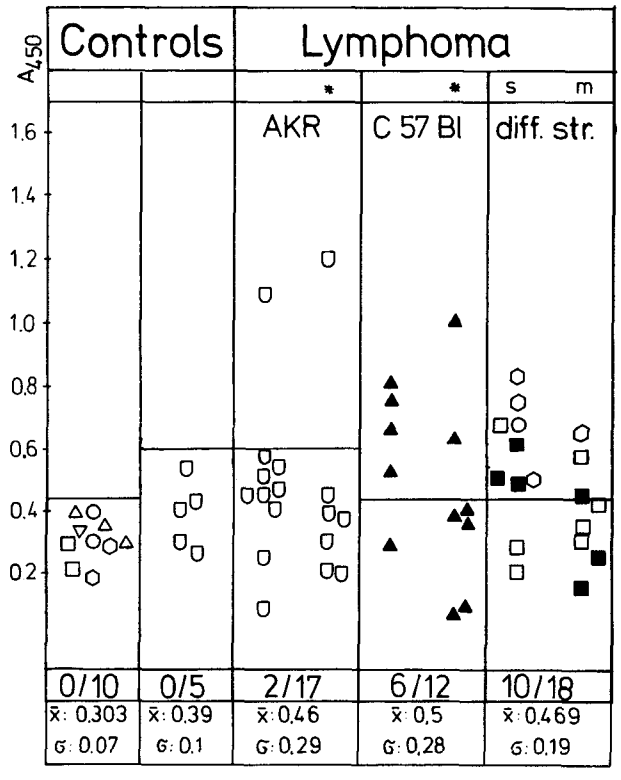

Fig. 2. AKR MuLV gp70 specific circulating immune complexes in the sera of lymphoma-bearing mice. For symbols see Fig. 1

(mean value 10 μg/ml). This was seen in lymphoma animals and in control mice (Fig. 3). Except for one mouse with lymphoma no AKR MuLV gp70 specific antibodies were detected in either the lymphoma group or the controls. Also no differences were noticed in one of the parameters between mice treated with alkyllysophospholipid (ALP) as an antitumor drug and their untreated counterparts.

II. X-Ray Induced C57Bl/6 T-Cell Lymphoma

The irradiated animals developed lymphomas 7–8 months after X-ray treatment. Only 2 out of 11 tumor-bearing mice had elevated values in the C1q-test for CIC. In contrast half of the mice (6 out of 12) with X-ray induced lymphomas showed increased levels of AKR MuLV gp70 specific CIC (Wilcoxon test significant against controls; Fig. 2). But there was a difference between lymphoma mice which had been pretreated with ALP and untreated mice. Less ALP-treated mice (two out of seven) exhibited specific CIC above normal than

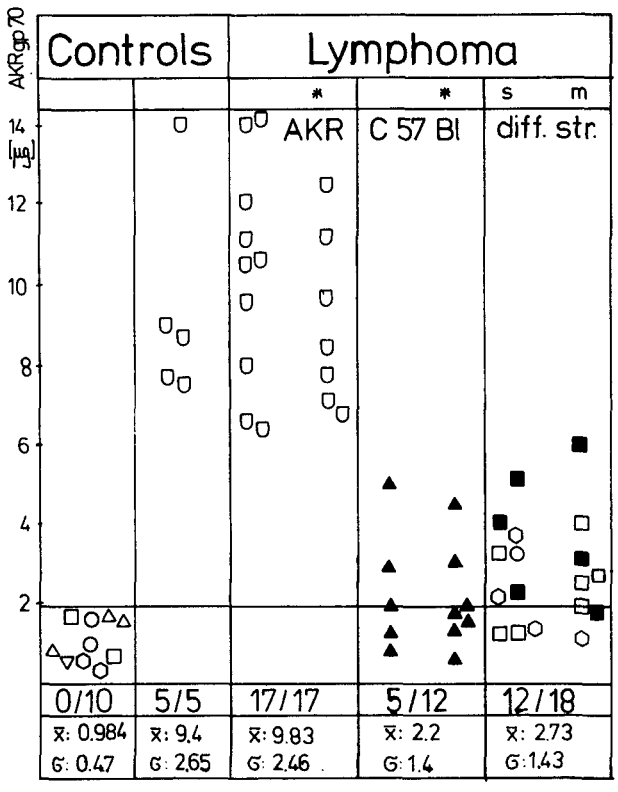

Fig. 3. AKR MuLV gp70 in the sera of lymphoma-bearing mice. For symbols see Fig. 1

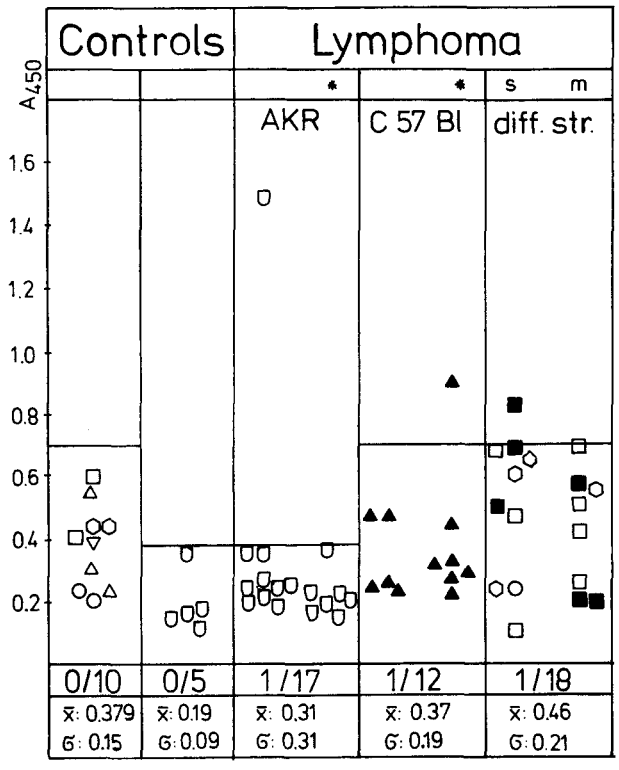

Fig. 4. Antibodies against AKR MuLV gp70 in the sera of lymphoma-bearing mice. For symbols see Fig. 1

untreated controls (four out of five). Also 5 out of 12 lymphoma animals had AKR MuLV gp70 serum levels above normal (2–6 µg/ml) (see Fig. 3). Only one mouse (ALP treated) with elevated AKR MuLV gp70 antibodies was detected. ALP-treated and untreated mice had an almost similar pattern concerning viral protein and viral antibodies.

III. Spontaneous Late B-Type Lymphoma

Spontaneous B-Type lymphomas (according to the classification of T. Dunn) appeared in the low leukemia mouse strains BALB/c, X/Gf, and CBA between the 20th and the 25th months of life. Some of the animals were suffering from a second malignancy (predominantly alveolar lung carcinomas) in addition to the lymphoma (see Figs. 1–4; column 5, m). A portion of the mice had been injected with ^{227}Th for osteosarcoma induction, but had not developed osteosarcoma.

Elevated C1q-binding CIC were generally not detected in this group of mice (1/18). In contrast, a significant number of animals (10 out of 18) were found to harbour AKR MuLV gp70 containing CIC above the background of healthy age-matched mice (Wilcoxon test significant). The same result was obtained for AKR MuLV gp70 antigen bodies (Figs. 2, 3). Twelve out of 18 lymphomatous mice had increased gp70 serum levels (2–6 µg/ml). The viral antibody titers remained in the normal range (see Fig. 4; column 5). No major differences were found between animals with only lymphoma (s) or with an additional second tumor (m).

D. Discussion

Circulating immune complexes detectable by a C1q-binding test can only rarely be observed in lymphoma-bearing mice. This is in some contrast to the situation with leukemias and lymphomas in men where about 30%–40% of the patients have a significant increase of C1q-binding CIC [2]. The possibility of a minor sensitivity of the test system used in our experiments could be ruled out by the detection of high

amounts of CIC in the serum of NZB mice known to develop an autoimmune disease accompanied by CIC formation (data not shown).

On the other hand antigen-specific (AKR MuLV gp70) CIC were present in the sera of C57Bl/6 mice with X-ray induced T-cell lymphomas and in the sera of low leukemia mice with late spontaneous lymphomas (T. Dunn type B). These CIC are obviously below the detection level of the C1q-binding test used. The presence of AKR MuLV gp70 specific CIC is accompanied by elevated AKR MuLV gp70 in the serum and normal antibody titer against AKR virus. This pattern clearly separates lymphoma animals from healthy individuals. Whether this situation indicates an antigen excess or antigen-antibody complexes with free antibody binding sites is unknown.

A different pattern has been observed from that described above in lymphoma-bearing AKR mice. Despite high serum levels of AKR MuLV gp70 no increased antibodies or CIC against this antigen were detected. The same situation was also found in healthy AKR mice. This describes a total immunotolerance of AKR mice against this endogenous viral glycoprotein. The anti-AKR MuLV gp85 antibody used in these experiments had a broad reactivity against ecotropic (Friend MuLV) and xenotropic (BALB virus: 2) murine retroviruses (G. Hunsmann, personal communication). It should therefore be reasonable to assume that all MuLV glycoprotein specificities which have been described in preleukemic and leukemic AKR mice [6] should have been detected.

We have no information on the prognostic value of the antigen-specific CIC in mice because the animals had been killed at the time of tumor detection. Our recent finding of a shorter survival time of those patients in CML blast crisis who had SiSV gp70 specific CIC or antigens in comparison to negative patients indicates such a possibility. This observation is in perfect accordance to survival data of patients in CML blast crisis with and without C1q-binding CIC [2]. The observation of CIC specific for retroviral antigens in murine and human leukemias and lymphomas suggests that retrovirus antigen-specific CIC in addition to their possible contribution to the course of the disease might be of prognostic value in these malignancies.

Acknowledgements

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References

1. Baldwin RW, Robins RA (1976) Factors interfering with immunological rejection of tumours. Br Med Bull 32:118–123
2. Carpentier NA, Louis JA, Lambert PH, Cerottini JC (1981) Immune complexes in leukemia and adult malignancies. In: Serrou B, Rosenfeld C (eds) Immune complexes and plasma exchanges in cancer patients. Elsevier, North Holland, pp 111–134
3. Day NK, Good RA, Witkin SS (1981) Circulating immune complexes and complement in human malignancy. In: Serrou B, Rosenfeld C (eds) Immune complexes and plasma exchanges in cancer patients. Elsevier, North Holland, pp 99–109
4. Erfle V, Hehlmann R, Schetters H, Schmidt J, Luz A (1981) Radiation-induced murine leukemias and endogenous retroviruses: the time course of viral expression. In: Neth, Gallo, Graf, Mannweiler, Winkler (eds) Haematology and blood transfusion, vol 26. Modern trends in human leukemia IV. Springer, Berlin Heidelberg New York, pp 537–540
5. Hehlmann R, Schetters H, Erfle V (1981) ELISA for the detection of antigens cross-reacting with primate C-type viral proteins (p30, gp70) in human leukemic sera. In: Neth, Gallo, Graf, Mannweiler, Winkler (eds) Haematology and blood transfusion, vol 26. Modern trends in human leukemia IV. Springer, Berlin Heidelberg New York, pp 530–536
6. Kawashima K, Ikeda H, Hartley JW, Stockert E, Rowe WP, Old LJ (1976) Changes in expression of murine leukemias virus antigens and production of xenotropic virus in the late preleukemic period in AKR mice. Proc Natl Acad Sci USA 73:4680–4684
7. Rossen RD, Reisberg MA, Hersh EM, Gutterman JU (1977) The C1q binding test for soluble immune complexes: Clinical correlations obtained in patients with cancer. J Natl Cancer Inst 58:1205–1215
8. Rossen RD, Zubler RH, Day NK, Reisberg MA, Morgan AC, Gutterman JU, Hersh EM (1978) Detection of immune complex-like materials in cancer patients sera: a compara-

tive study of results obtained with the C1q deviation and C1q binding tests. J Lab Clin Med 91:191–204
9. Schetters H, Hehlmann R, Erfle V (1981) ELISA for the detection and quantification of C-type viral glycoprotein (gp70) using antibodies that recognize the protein moieties of the glycoproteins. J Virol Methods 2:357–366
10. Sodomann CP, Schmidt H, Havemann K. (1979) Zirkulierende Immunkomplexe bei Patienten mit malignen Lymphomen. Blut 38:435–446
11. Theofilopoulos AN, Andrews BS, Urist MM, Morton DL, Dixon FJ (1977) The nature of immune complexes in human cancer sera. J Immunol 119:657–663
12. Wehler C, Andrews JM, Bing DH (1981) The use of solid phase Clq and horseradish peroxidase-conjugated goat anti-IgG for the detection of immune complexes in human serum. Mol Immunol 18:157–162