IMMUNOLOGY

Differentiation between Viral and New Cellular Antigens in Graffi Leukaemia of Mice

MYELOID Graffi leukaemia induced by a virus in mice contains both leukaemia virus and virus-induced antigens[1,2]. The presence of leukaemia virus can be demonstrated by cell-free transmission of the disease to newborns and by the ability of the virus to induce the production of antibodies in adults (MAP test) against antigens appearing on the surface of leukaemia cells[3,4]. Specific antibodies have been shown by the cytotoxic test in the presence of complement and by the indirect fluorescent–antibody technique using living cells as targets[2]. Virus-induced surface antigens of leukaemia cells can be detected by the same techniques. *In vivo*, syngeneic inocula of leukaemia cells are rejected in Graffi immune animals, which suggests the presence of specific surface antigens, too. For tumours induced by polyoma, *S V* 40, adenovirus, and Rous virus there is evidence that the specific surface antigens represent new cellular antigens unrelated to viral antigens[5,6]. This last problem, however, is still unsolved for virus-induced leukaemia of mice. Cells of primary and transplanted leukaemias are continuously producing virus by budding it from the cell surface. It is therefore conceivable that the virus-induced surface antigens consist of both viral and new cellular antigens. On the other hand, Landschütz sarcoma 1 cells, which naturally harbour the Graffi virus[7], were found to be resistant to the specific rejection response *in vivo* and to cytotoxic antibodies *in vitro*[8]. These cells, however, reveal a weak but distinct punctiform membrane fluorescence in the indirect fluorescent antibody test with Graffi hyperimmune sera of mice[8]. Provided the positive fluorescence reaction with sarcoma cells is solely the result of the presence of virions or viral antigens on the cell surface, then a differentiation between viral and new cellular antigens of leukaemia cells must be possible. To substantiate this hypothesis the following experiments were carried out. Landschütz sarcoma 1 cells were used to absorb a Graffi immune serum pool containing virus-neutralizing antibodies and antibodies against specific surface antigens of leukaemia cells. Usually 0·1 ml. samples of the serum were added to cell pellets containing 80 million living cells. The mixture was then incubated for 60 min at 37° C. After centrifugation the supernatant serum was again absorbed with another pellet of sarcoma cells. After two absorptions the serum was tested against syngeneic *L* 414/2*a* Graffi chloroleukaemia cells using the fluorescent antibody technique. Its virus-neutralizing capacity was subjected to the MAP test.

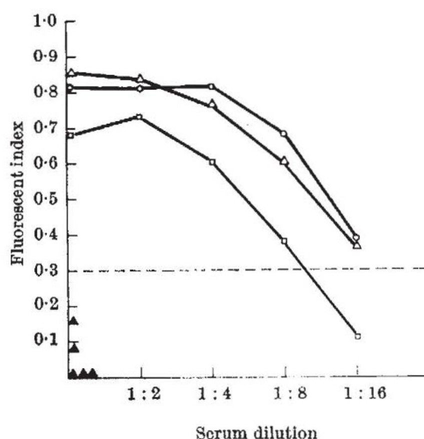

Fig. 1. Titration of a Graffi immune serum pool using *L* 414/2*a* Graffi chloroleukaemia cells as targets. Results of the indirect fluorescent antibody test. Graffi immune serum pool: ○, unabsorbed; △, absorbed with normal spleen and lymph node cells; □, absorbed with Landschütz sarcoma 1 cells; ▲, absorbed with *L* 414/2*a* leukaemia cells. Fluorescent index (FI) is the percentage of unstained cells in control serum minus percentage of unstained cells in immune serum divided by percentage of unstained cells in control serum. FI≧0·3 was arbitrarily regarded as positive.

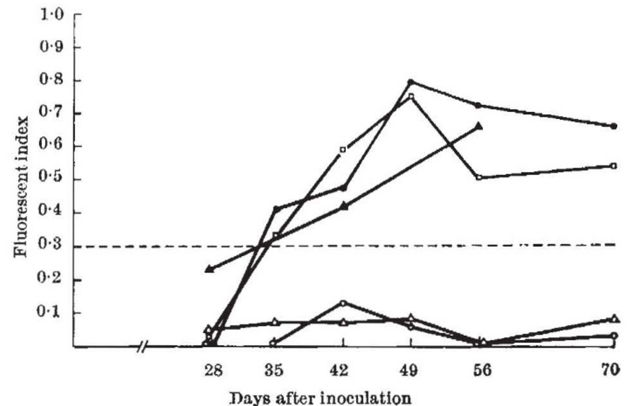

Fig. 2. Antibody response against Graffi *L* 414/2*a* target cells after inoculation of adult XVII/*Bln* mice with a cell-free Graffi leukaemia filtrate incubated with absorbed and unabsorbed Graffi immune serum *in vitro*. Antibody response after inoculation of filtrate plus: ○, unabsorbed Graffi immune serum; △, Graffi immune serum absorbed with normal spleen and lymph node cells; □, Graffi immune serum absorbed with Landschütz sarcoma 1 cells; ▲, Graffi immune serum absorbed with *L* 414/2*a* leukaemia cells; ●, normal mouse serum. For FI see Fig. 1.

As positive and negative controls, we used the same serum pool, but unabsorbed or absorbed with *L* 414/2*a* leukaemia cells or normal spleen and lymph node cells. Fig. 1 shows that the titre of the immune serum pool, as measured by the fluorescent index, did not decrease after two absorptions with normal cells and decreased only slightly after absorption with sarcoma cells. There was a complete elimination of antibodies after absorption of the serum pool with *L* 414/2*a* Graffi leukaemia cells. To test the virus-neutralizing activity, a cell-free filtrate of a primary Graffi leukaemia diluted 1 : 100 (w/v) was mixed at a ratio of 1 : 10 with the sera to be tested. The final concentration of the filtrate was 1 : 1,000. After 60 min of incubation at 37° C, 0·2 ml. samples of the mixture were injected intraperitoneally into adult XVII mice. At weekly intervals blood was taken from the animals by puncturing the retro-orbital sinus. The appearance of serum antibodies against Graffi leukaemia cells was controlled by the fluorescence test. The results of this experiment are shown in Fig. 2.

Sarcoma 1 cells obviously eliminate virus-neutralizing antibodies from the Graffi immune serum pool. There were no differences in the antibody response as compared with those groups of animals inoculated with filtrate plus normal serum or filtrate plus immune serum absorbed with Graffi leukaemia cells. The MAP test became positive about 5 weeks after inoculation of the material. In contrast, the MAP test was negative after injection of filtrate incubated with unabsorbed Graffi immune serum or immune serum absorbed with normal spleen and lymph node cells. In another experiment with a Graffi virus-superinfected Gross leukaemia, which shows the same immunological characteristics as sarcoma 1 cells[8], the data of the absorption tests presented in this paper have been confirmed. Whereas the original Gross leukaemia cells did not show any absorption effect on a Graffi immune serum, virus-neutralizing antibodies were completely eliminated by the cells superinfected with Graffi virus.

In summary, Landschütz sarcoma 1 cells, which naturally harbour the Graffi virus, were shown to eliminate virus neutralizing antibodies from a Graffi immune serum pool without absorbing antibodies against surface antigens of leukaemia cells. The leukaemia cells absorb both virus-neutralizing antibodies and antibodies against

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surface antigens of leukaemia cells from the immune serum, and so virus-induced surface antigens of leukaemia cells appear to consist of both viral and new cellular antigens.

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Suppression of Synthesis of Allotypically Defined Immunoglobulins and Compensation by another Sub-class of Immunoglobulin

AMONG the light chains of the rabbit immunoglobulin molecules four allotypically determined molecular species can be distinguished: A4, A5, A6[1] and A9[2]. These specificities are determined by allelic genes at the A_b allotypic locus[1,2]. Thus a rabbit homozygous at this locus synthesizes only one of these specificities, but a heterozygous rabbit synthesizes two. The phenotypic expression of the synthesizing capacity of heterozygotes can be altered by administration of anti-allotype antibody; synthesis of immunoglobulins of the paternal type can be inhibited ("suppressed") either by uterine transfer[3-7] of allotype antibody from the mothers immunized against the allotypic specificity of the prospective father[8] or by injecting the young rabbits with allotype antiserum during the first few days of life[9-11]. When a heterozygous rabbit is exposed to anti-allotype serum the resulting suppression of one allotypic specificity is compensated by increased synthesis of the product of the second allele; thus the total concentration of IgG is not affected and is similar to that of the normal rabbits[9,11].

I have suggested previously that the suppression is caused by death of such cells as were committed to the synthesis of immunoglobulins carrying the respective allotypic specificity[11]. If this were the case, the compensation in immunoglobulin synthesis in suppressed rabbits would be caused by compensatory proliferation of cells committed to the synthesis of the other allotype. The question arose as to whether suppression could be induced in homozygotes and, if so, whether it would be accompanied by a compensatory increase of other immunoglobulins.

The uterine transfer of immunoglobulin causes large concentrations of maternal immunoglobulins in the serum of the newborn rabbits. This circulating immunoglobulin would prevent anti-allotype antibody from reaching the lymphoid cells. In order to circumvent these difficulties, conditions were created in which the foetal rabbits did not acquire maternal immunoglobulins of the allotypic specificity which we were going to suppress in the offspring. This was achieved by using a heterozygous A^4/A^5 female in which the synthesis of A5 was suppressed by injection of the corresponding antibody at birth. This rabbit was then mated with a homozygous A^5/A^5 male; A5 remained undetectable in the circulation of the pregnant animal. Her offspring were divided into two groups: four rabbits were left untreated and the three remaining rabbits were each given 4·3 mg of anti-A5 N during the first 8 days of life. (The amount of antibody N in anti-allotype serum was estimated by measuring nitrogen increment of cellulose immunoabsorbent after incubation with antiserum as described before[10].) Both groups of rabbits were bled at weekly intervals and the relative concentrations of allotypically defined immunoglobulins (A4 and A5) as well as the concentration of the IgG were measured by single diffusion[12] as described earlier[10]. The antisera used for this assay were rabbit anti-A4 and anti-A5, and goat anti-rabbit gamma-globulin. To interpret the results it was necessary to know the genotypes of all the offspring from the mating between the "suppressed" A^4/A^5 mother and the A^5/A^5 father. Clearly, the offspring could be either A^4/A^5 or A^5/A^5. We could distinguish between these two alternatives by the synthesis of A4 which would, of course, only occur in animals the genotype of which was A^4/A^5.

Fig. 1*A* shows the concentrations of A4 as a function of time in the untreated rabbits and in a homozygous rabbit injected with anti-A5 serum. Immunoglobulins of A4 specificity were detectable for 32 days in the untreated and for 47 days in the injected animals. In both cases the half-life was practically the same (about 5 days). The longer period, during which the A4 immunoglobulin could be demonstrated in the injected animal, was attributable to the large amounts of A4 which were introduced with the anti-A5 serum.

We have thus seen that homozygous animals could be identified by the steadily declining concentration of A4 immunoglobulins. To examine the overall changes in IgG, I next measured the concentration of that protein as a function of time (Fig. 1*B*). Again a decline in concentration during the first 20–32 days was observed, but, unlike A4, the concentration of IgG increased after this initial decline. As a consequence of the injections of anti-A5 serum, the concentration of IgG in the serum of the injected rabbit was initially higher than in the uninjected homozygous littermates, but after 38 days the differences disappeared, and the rate of increase was the same in both the injected and untreated animals. It follows that the overall rate of synthesis of the total IgG in the injected rabbit was not affected by the treatment.

In marked contrast to the synthesis of IgG, the synthesis of allotypic A5 immunoglobulin was considerably affected in the injected rabbit. This is illustrated in Fig. 2 which compares the concentrations of A5 in the injected and untreated animals. In the uninjected animals A5

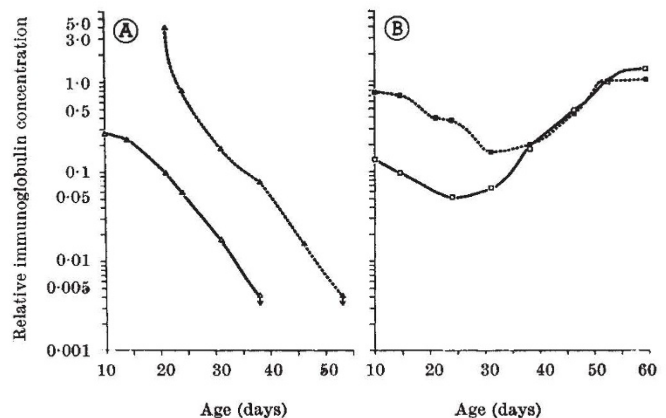

Fig. 1. Concentrations of A4 and IgG in A^5/A^5 rabbits as a function of time. The rabbits were born as a result of a mating: male A^5/A^5 × female A^4/A^5. The synthesis of A5 in the mother was "suppressed" by the neonatal injection of anti-A5 serum in order to prevent A5 from being transferred to her offspring. (*A*) Elimination of passively acquired A4 immunoglobulin. - - ▲ - -, Concentrations of A4 in homozygous A^5/A^5 rabbit injected with anti-A5 serum; —Δ—, average concentrations of A4 in three untreated homozygous littermates of the above rabbit; ♠, A4 immunoglobulin undetectable by single diffusion method. (*B*) Elimination and synthesis of IgG. - - ■ - - , Concentrations of IgG in A^5/A^5 rabbit injected with anti-A5 serum; —□—, average concentrations of IgG in three untreated A^5/A^5 littermates of the above rabbit.

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