Induction of Antinuclear Antibodies in (C × B6) F1 Mice Inoculated with Graffi and Rauscher Leukemogenic Viruses (36936)

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Viruses have often been implicated in the etiology of autoimmune manifestations observed in man (1) or in animal models such as mice (2) or dogs (3). Tonietti, Oldstone and Dixon (4) have shown that, in New Zealand mice, polyoma and lymphocytic choriomeningitis viral infections induce an abnormally high incidence of antinuclear antibodies detectable by immunofluorescence (ANF). According to Mellors, Aoki and Huebner (2) the type C viruses immunologically related to the Gross leukemia virus, which are naturally present in NZB mice, are responsible for the autoimmune diseases observed in this strain. Nevertheless their ANF inducing capacity has not been demonstrated. Confirming a preliminary experiment (5) we report in the present paper that inoculation with two different RNA oncogenic viruses actually leads to the appearance of ANF in (BALB/c × C57/Bl6) F1 mice (C × B6).

Materials and Methods. C57/Bl6, BALB/c and F1 hybrids obtained from our colonies were maintained under specific pathogen-free environment up to their first inoculation. Graffi virus, kindly provided in 1962 by Professor Graffi, is regularly maintained by acellular transmission in C57/Bl 6 mice and Rauscher virus (provided by Dr. F. J. Rauscher in 1963) in BALB/c. Acellular extracts from spleen, tumors or blood were prepared as previously described (6) and stored at −170° in liquid nitrogen.

Two samples of Graffi virus (G 96 and 77), differing in their oncogenic potency (as judged by the incidence and latency of tumors in C57 and C × B6 mice) and two different dilutions of a sample of Rauscher virus (RLV 396) were used in this study.

Inactivation of these viruses was obtained by ultraviolet light (uv) exposure from 60 to 120 mn at room temperature as described in (7). Specific neutralization of the Rauscher virus was performed by incubating for 45 min at room temperature and 45 min at +4° a 10^{-3} dilution of RLV 396 with two different anti-MuLv antisera (8), namely C57/Bl6 anti-RLV serum prepared as in (8) and W/Fu rat anti-C 58 NTD serum prepared according to (9), using concentrations known to induce a 2 log dilution of the oncogenic capacity of RLV 396 as shown by the drop in the incidence of tumors obtained in BALB/c mice. Normal spleen cells were obtained from 6 wk old BALB/c and C57/Bl 6 and killed by sonication or by repeated freezing and thawing. Mice were inoculated intraperitoneally either as newborns with the normal killed cells or the Graffi viral preparations or at 6 wk of age with the RLV preparations. Control and inoculated mice were examined weekly and leukemias were checked by cytology and histology. All mice were tail bled at regular intervals and their sera were tested for ANF antibodies by indirect immunofluorescence as previously described (10) using rat liver sections as a substrate. Absorption studies were performed for 1 hr at 37° and 48 hr at 4° on pooled ANF positive sera using 500 μg/ml of native and heat denatured DNA (Worthington) and of calf thymus nucleoproteins (NP) prepared as in (11). Enzyme treatment of the rat liver sections was performed as in (11) using desoxyribonuclease (DNase), ribonuclease (RNase) (both from Worthington) and trypsin (Nutritional Biochemicals Corp.).

Results. Table I shows that ANF incidence

TABLE I. ANF and Tumor Incidence in C × B 6 Mice Inoculated with Graffi and Rauscher Viral Preparations.

Inoculum	No. tested	% ANF + time after inoculation: 2 mo	6 mo	% Tumors at age: 10 mo	16 mo
C × B 6 controls	48	0	4.5	0	0
+10.7 killed BALB/c spleen cells	26	0	—	—	—
+10.7 killed C 57/Bl 6 spleen cells	30	0	—	—	—
Graffi virus					
—G 96: 0.1×10^{-1}	46	0	45	0	30
—G 77: 0.1×10^{-1}	20	50	58	30	—
—G 77: 0.1×10^{-1} × uv 1 hr	14	14	37	14	—
Rauscher virus (RLV 396)		2 mo	4 mo	3 mo	
-0.1×10^{-1}	18	92	—	100	
-0.1×10^{-1} × uv 2 hr	14	0	7	0	
-0.1×10^{-3}	17	47	71	0	
-0.1×10^{-3} × uv 2 hr	12	0	0	0	
-0.1×10^{-3} + anti-RLV	14	0	—	0	
-0.1×10^{-3} + W/FU anti-C 58 NTD serum	15	0	—	0	

is significantly higher in Graffi and Rauscher inoculated mice than in non-inoculated controls. It shows moreover that ANF were not detected after 2 mo in F1 mice inoculated with normal disrupted spleen cells or with specifically neutralized RLV. A striking correlation exists between the leukemogenic potency of a given viral preparation (Graffi or RLV) and its ability to induce ANF. ANF appear at an earlier stage when mice are inoculated with the most leukemogenic Graffi virus (G 77 compared to G 96) or with the higher concentration of RLV. After uv exposure the incidence of leukemias and of ANF drops at the same rate. However ANF induction is not directly related to the appearance or the growth of leukemias since many tumor bearing mice are ANF negative and many ANF positive mice have no tumors (as seen after inoculation of a 10^{-3} RLV dilution). On the other hand the role of a persistent viral infection in ANF induction is suggested by the higher incidence of ANF in late (4 to 6 mo) than in early samples.

The titers of positive reactions were low or moderate: the mean titer of early samples was 1/8 compared to 1/32 for the later samples. The immunofluorescence pattern of positive reactions was in all cases homogeneous. Such patterns are usually considered to reflect the presence of serum antibodies with NP and/or DNA specificity (4, 12).

The results of absorption studies and of enzyme pretreatment of rat liver sections, performed to determine if specific staining could be abolished by these reagents are reported in Table II. These data show that at least the late samples have NP specificity since ANF activity of pooled positive sera was almost completely abolished by absorption with NP and since the homogeneous staining of nuclei by ANF positive sera was prevented both by DNase and by trypsin pretreatment. On the other hand the drop in titer and intensity following absorption with denatured DNA or pretreatment with RNase is too weak to allow definite conclusions as to a possible RNA and/or denatured DNA specificity of ANF positive sera.

Discussion. These results indicate that inoculation with Rauscher and Graffi viral preparations can induce or trigger the appearance of antinuclear antibodies in mice. Since our viral preparations are not purified leukemogenic particles, they also contain cellular proteins and may be contaminated by

TABLE II. Effect on ANF Staining of Absorption and Enzyme Studies.

Substrate	Enzyme	Nature of serum: pooled ANF positive sera of late samples (mo)	Absorption	Final dilution of serum	Intensity at a ½ dilution
Rat liver sections	—	4–6	—	1/32	+++
	—	4–6	NP	1/2	±
	—	4–6	Native DNA	1/32	+++
	—	4–6	Denatured DNA	1/16	++
	DNase	4–6	—	NT	±
	Trypsin	4–6	—	NT	—
	RNase	4–6	—	NT	+

other viruses. However the ANF inducing role of cellular proteins is ruled out by the absence of ANF after inoculation of normal disrupted cells of the same strains used for maintaining the viruses.

The presence in our preparations of small amounts of pathogenic viruses such as LCM or polyoma (known to induce ANF in New Zealand mice) (4), or of latent nonpathogenic viruses such as Mycoplasma cannot be excluded. However the parallelism observed between the leukemogenic potency and the ANF inducing capacity of several unrelated viral preparations points to the actual role of Graffi and RLV in ANF induction. This is strongly supported by the absence of ANF after inoculation with specifically neutralized RLV.

Unlike New Zealand mice, (C × B6) F1 and their parental strains do not spontaneously develop autoimmune phenomena and have a low or moderate incidence of spontaneous ANF. Our results, together with those observed in New Zealand mice after polyoma and LCM inoculation (4) thus suggest that ANF induction by viruses could well be a widespread phenomenon which may not be restricted to a few viruses or to very unusual genetic predisposition. Moreover the fact that leukemogenic viruses known to be latent in numerous strains of mice are able to induce ANF points to the possible role of such viruses in the appearance of antinuclear antibodies either spontaneously or after murine tissue inoculations.

The relationship between RNA viral infections and induction of antinuclear antibodies with at least partial NP or DNA (13) specificity is not yet understood. Factors known to lead to the appearance of autoimmunity can act by either central or peripheral mechanisms or both. In the case of our viruses a direct action on the central mechanisms of self recognition could occur since leukemogenic oncona viruses are able to multiply in lymphoid cells and have been shown to act as immunosuppressive agents (14). On the other hand viral multiplication could act through various peripheral mechanisms. The release of unusual amounts or kinds of immunogenic host nuclear antigens is unlikely since Rauscher and Graffi viruses have no cytolytic effect *in vitro* (15). Viral RNA and ribonucleoproteins are produced in large amounts in type C virus infected mice and this could provide the necessary immunological stimulus since it has been shown that inoculation of New Zealand mice with viral or synthetic RNA induces not only RNA but DNA antibodies (13). The DNA copies of viral RNA which may be produced during viral multiplication (16, 17) could also act as immunogens. Further studies are now in progress to test these different hypotheses.

Summary. Antinuclear antibodies were shown to appear in (C × B6) F1 mice after inoculation with Graffi and Rauscher leukemogenic viruses. Their incidence is correlated with the infectivity of the viral inoculum. The mechanism of ANF induction by viral preparations and the possible role of leukemogenic viruses in the autoimmune pathology of mice are discussed.

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