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Antibody and Antigen Levels in Rats Inoculated with Moloney Sarcomas and Viral Antigens

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Abstract—*Antibody to p30 or gp70 as well as p30 antigen was measured in the serum of animals immunized with moloney sarcoma cells and then challenged with a tumor. Untreated animals exhibited first undetectable and then increasing levels of antibody throughout a* 32-day *period. Immunized rats had high levels of antibody prior to and after tumor challenge. Serum levels of p30 increased sharply during the period when progressive tumor growth usually occurs. During this period, conditions are favorable for the formation of antigen–antibody complexes which may effect tumor growth. The data suggest that other factors also influence the growth ot tumors in rats.*

INTRODUCTION

WE HAVE previously reported that growth of moloney sarcomas in rats correlates with antibody responses to the viral core protein p30, and that immunization with p30 enhances the growth of these tumors [1, 2]. Recently, considerable interest has emerged concerning the role of circulating immune complexes in tumor bearing animals and in human patients [3–6]. Indeed, in the earlier studies, one explanation offered was that p30–anti-p30 complexes might exhibit immunoinhibitory effects. Subsequently, it has been reported [7] that rats carrying moloney sarcomas do exhibit immune complexes in the circulation. Two additional types of experiments are needed to complement these studies: (a) a simultaneous measurement of the levels of antibody and of antigen during tumor growth to establish whether prevailing conditions favor the formation of complexes; (b) a direct test of the effect of inoculating performed immune complexes on the rate of tumor growth. The present report is concerned with an approach to the first type of experiment.

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MATERIALS AND METHODS

Tumor cells, virus and viral antigens

Moloney sarcoma cells (MST), moloney leukemia virus (MULV) and viral p30 and gp70 antigens were prepared by methods described previously [2, 8]. MST cells were derived from Brown Norway (BN) rats and produce MULV *in vitro* and *in vivo* [8].

Animals and immunization

BN rats were obtained from our breeding colony using stock provided by Scripps Clinic and Research Foundation, La Jolla, California. Groups of 8–10, 3-month-old female rats were immunized with 3 footpad and i.v. injections of 10 μg p30 or gp70 [2] or with s.c. doses of 5×10^5 MST cells (a threshold dose that will regress). One week after the last injection, these groups and non-immunized groups were challenged with 2×10^7 MST cells s.c. Blood samples were collected by tail bleedings every 4–6 days.

Radioimmunoassays

Antibody to p30 or gp70 was measured by incubating 5 μl serum with 0.1–0.2 ng ^{125}I labeled antigen (20–30 μCi/μg, >80% precipitable in 10% TCA) for 30 min at room temperature. Rat immunoglobulin with bound antigen was precipated by adding 10

mg of a gamma globulin fraction of hyperimmune goat anti-rat gamma globulin [2]. Precipitates were washed with phosphate buffered saline by centrifugation and radioactivity was measured. Antibody activity of unknown serum was related to reference controls of hyperimmune BN anti-MST [1, 2] and normal BN serum.

p30 Was measured by a competitive inhibition radioimmunoassay [2, 9] using standards of isolated MULV p30 and rat anti-p30.

RESULTS

Antibody responses to p30 and p30 levels

Figure 1a shows antibody responses to p30 in rats immunized with MST and in non-immunized rats following challenge with MST. Figure 1b shows the levels of p30 in the

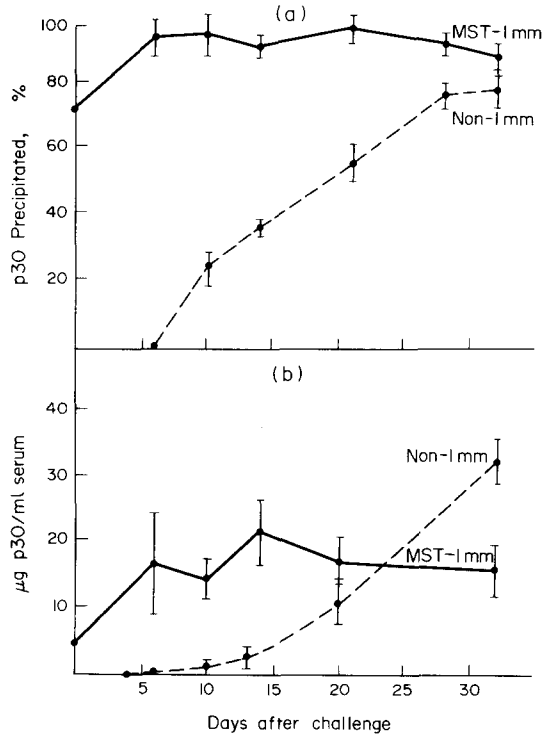

Fig. 1. Antibody to p30 (a) and serum p30 levels (b) in BN rats challenged with MST. Values are a percentage of 0.1–0.2 ng *input p30 precipitated specifically by* 5 μl *serum (a) and* μg *p30/*ml *serum* × 10 *(b), average of* 8–10 *animals* ± S.E. *(vertical bars). MST-Imm = immunized with subthreshold doses of MST prior to challenge (these rats are resistant to tumor growth). All rats challenged on* day 0 *with* 2×10^7 *MST cells s.c.*

serum of these same animals. Immune rats contained high levels of antibody at the time of tumor challenge and the levels remained elevated throughout the period of observation. In non-immune rats, antibody was first detected at 10 days and increased throughout a 32 day period.

The serum of animals immunized with MST contained detectable p30 at the time of tumor challenge (1b). Since the MST cells used for immunization were producing virus particles, these animals presumably developed some degree of viremia. Non-immune rats exhibited no detectable p30 at the time of tumor challenge. p30 was detected in these animals at day 14 but increased significantly after day 20. The levels of p30 may actually have been higher than the measurements indicate since some antigen would be bound by antibody and/or cleared from the circulation.

Immune complexes would be predicted to occur in the sera of both groups of animals and we have been able to demonstrate complexes by Raji cell assays, but we have not determined what proportion of total antigen is complexed.

Antibody responses to p30 and gp70

Antibody responses to p30 and to gp70 in several other groups of rats challenged with MST were also measured (Table 1). In non-immunized rats, antibody to gp70 appeared more slowly and attained lower peak levels following MST challenge. Immunization with gp70 did not significantly affect a subsequent response to p30. Immunization with p30, on the other hand, augmented the responses to gp70 in tumor bearing rats presumably because immunization with p30 stimulates tumor growth [2]. In animals immunized with p30 or gp70 and not challenged with MST, antibody levels slowly declined over the period of observation. p30 antigen could not be detected in the sera of these latter animals.

DISCUSSION

Immunization with moloney sarcoma cells or with moloney leukemia virus antigens will influence the growth of moloney sarcomas. BN rats immunized with subthreshold doses of MST cells are resistant to a challenge of MST while animals immunized with p30 exhibit an enhanced rate of tumor growth [2]. The growths of MST in the rats used in the present studies were comparable to those of similarly treated rats reported previously [2]. In non-immune BN rats a threshold dose of MST will grow progressively in most animals after 20 days. While previous studies indicated a correlation of tumor growth with antibody

Table 1. Antibody in serum of tumor bearing BN rats

Immunized with*	Test antigen	Percent of antigen precipitated† on: day 0*	day 10	day 20	day 30
non-immune	p30	0	25	54	78
MST	p30	75	96	97	92
p30	p30	74	90	95	94
gp70	p30	0	35	64	81
non-immune	gp70	0	2	20	28
MST	gp70	13	53	53	45
p30	gp70	0	3	40	55
gp70	gp70	15	50	75	84

*All animals challenged with 2×10^7 MST s.c. Day 0 is day of challenge, others are days after challenge.

†Per cent of input of 0.1–0.2 ng ^{125}I labeled p30 or gp70 co-precipitated by 5 μl serum. Means of 8–10 samples. Standard errors of measurements were 4–10%. Values corrected for trapping by non-immune serum (1–3% with p30, 3–5% with gp70).

responses to p30 [1] the present studies suggest that the period of progressive tumor growth may also correlate with serum levels of p30 antigen. During that period conditions are favorable for the formation of circulating immune complexes. Jennette and Feldman [7] have measured circulating immune complexes in BN rats bearing moloney sarcomas. They found the levels of complexes to be greatest during the interval of 20–40 days after tumor challenge. During this interval, we found increasing levels of antibody to p30 and a sharp rise in levels of p30 antigen. These observations are consistent with the possibility that immune complexes have an inhibiting effect on host immunity. These data complement those of Jennette and Feldman [7] since they did not identify the antigen components of the putative complexes. They did observe that levels of complexes would reach a peak and then decline in some animals during progressive tumor growth. We find that antigen levels may continue to rise after antibody appears to have leveled off. This would tend to reduce the size of complexes and make them more difficult to detect by Raji cell assays.

Obviously factors other than immune complexes affect tumor growth. Animals immunized with MST and resistant to tumor challenge would also develop complexes. These animals also exhibit greater levels of cellular immunity [10] and they contain antibody to tumor specific (TSA) antigens [11]. In contrast to the observation with p30, we have found that rats with regressing moloney sarcomas exhibit greater levels of antibody to TSA than do animals with progressing tumors (unpublished observations).

It may seem paradoxical that antibody and antigen could be measured in the same serum sample. However, the *in vitro* radioimmunoassays involve a dilution of the samples which may favor dissociation of complexes. In addition, if an animal were viremic a portion of the virus might become disrupted during the *in vitro* assay and release internal p30 antigens. Finally, an immune complex can contain unoccupied antibody binding sites and free antigenic determinates that would be available to react. A simultaneous occurrence of measurable virus and anti-viral antibody has also been reported by other investigators [12].

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