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# EFFECTS OF VARIOUS TREATMENTS ON THE DISTRIBUTION IN RATS OF IMMUNE COMPLEXES CONTAINING ANTIGENS OF MuLV LEUKEMIA VIRUS

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## ABSTRACT

Immune complexes (IC) containing antigens of MuLV ( $^{125}\text{I}$ p30-anti-p30 or  $^{125}\text{I}$ gp70-anti-gp70) and formed *in vitro* or *in vivo* were sequestered primarily in the spleen. Treatments of rats with trypan blue, known to inhibit the reticuloendothelial system (RES) and diethylstilbestrol (DES), known to stimulate the RES, resulted in slight but not significant reduction of uptake of IC by the spleen. Splenectomy did not increase sequestration of IC by the liver nor did it change the distribution of IC in other tissues. Protein A, a cell wall protein of *Staphylococcus aureus*, when injected with IC resulted in a 10-fold reduction of uptake of IC by the spleen. This effect was seen at 4 hours, 1 day, and 8 days after injection. Protein A did not change the binding of antigen to its respective antibody. The mechanism whereby Protein A exerts its effect is discussed.

## INTRODUCTION

Circulating immune complexes (CIC) have been implicated in the pathogenesis of several diseases including SLE, thyroiditis, glomerulonephritis, and cancer (Heir et al., 1977; Zanetti et al., 1977; Kalderon and Bogaars, 1977). Removal of these CIC could prove beneficial to patients. Terman et al. (1981) and Bansal et al. (1978) have shown that removal of CIC by extracorporeal perfusion of plasma from cancer patients over a paste of *Staphylococcus aureus* containing Protein A improved the conditions of these patients. We report here the effects of several treatments on the distribution in rats of IC containing antigens of murine leukemia virus (MuLV).

## MATERIALS AND METHODS

**Animals:** Brown Norway (BN rats, 3-6 months old, were obtained from our breeding colony and from Charles River Breeding Co. They were maintained on Purina Chow and water ad libitum.

**Antigens and Antibodies:** p30 and gp70 are core and viral envelope polypeptides of MuLV, respectively. They were labeled with I-125 using Chloramine T (McConahey and Dixon, 1966). Antibodies (BN anti-MST) to these antigens were formed in BN rats after several subcutaneous injections of subthreshold doses of Moloney sarcoma cells which express p30 and gp70. Antibody titers were assessed by radioimmunoassay (Jones et al., 1977).

**Formation and Administration of IC:** Immune complexes were made at equivalence by incubating 100 $\mu\text{l}$  of BN anti-MST with 20 ng of  $^{125}\text{I}$ -p30 or  $^{125}\text{I}$ -gp70 for 30 minutes at room temperature. Immune complexes thus formed were injected alone into normal BN rats or were coupled to 500 $\mu\text{g}$  of Protein A (Forsgren and Sjoquist, 1978; Sjoquist et al., 1972) for 15 minutes at room temperature before injection. A group of tumor bearing BN rats that had high antibody titer to p30 antigen was injected with 20 ng of  $^{125}\text{I}$ -p30. Animals were sacrificed 24 hours later unless otherwise indicated, and their body organs were collected, weighed and the radioactivity measured in a gamma counter. Blood ratios of these organs were calculated by dividing cpm/g of organ by cpm/g blood. Control groups of animals received the antigens in normal BN serum or saline.

To assess the effect of other treatments on the distribution of IC, BN rats were injected i.p. with trypan blue (RES depressant) suspended in PBS (50 mg at minus 24 hours and 10 mg at minus 4 hours to each animal) before injection of IC (Haskill et al., 1979). Other groups were injected s.c. with diethylstilbestrol (RES stimulant) dissolved in 95% ethanol (100 mg DES/Kg body weight) 3 days before injection

of IC (Warr and Slijvic, 1973). Other groups were splenectomized under ether anesthesia 2 days before injection of IC.

## RESULTS AND DISCUSSION

Immune complexes that formed *in vivo* when  $^{125}\text{I}$ -p30 was injected into immune tumor bearing BN rats were found to be sequestered in the spleen at one and 9 days later (Figure 1). Other tissues did not exhibit a significant uptake. The experiments described below represent attempts to alter this distribution using IC formed *in vitro* and injected into normal rats.

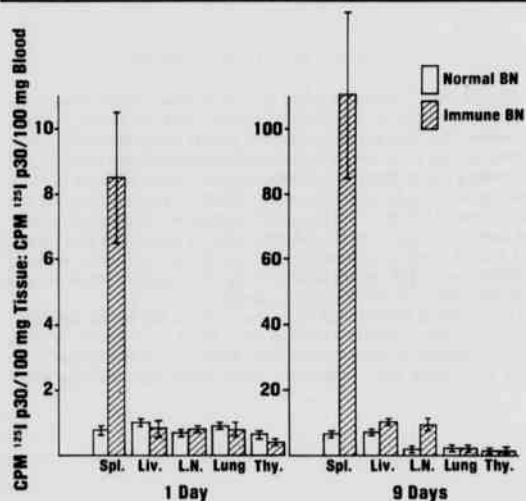


Figure 1. Distribution of  $^{125}\text{I}$ -p30 after injection into tumor bearing (immune) or normal BN rats. p30 (100 ng, approximately  $2 \times 10^6$  CPM) injected i.v. 15 days after s.c. injection of  $5 \times 10^6$  MST cells, or into untreated BN rats. Columns indicate average of 2 to 5 rats ( $\pm$  range or standard error). Tissue/blood ratios shown for spleen, liver, cervical nodes, lung and thymus; samples collected 1 or 9 days after  $^{125}\text{I}$ -p30 injection.

Treatment with trypan blue (RES depressant) or DES (RES stimulant) reduced slightly the uptake of IC by spleen as shown in Table 1. Neither agent altered significantly the overall tissue distribution of IC. In each case, the spleen exhibited the greatest uptake of IC per unit weight. Splenectomy did not increase the sequestration of IC by the liver and the absence of the spleen did not change significantly the distribution of IC in other tissues (Table 1).

Sequestration of IC containing p30 or gp70 in spleens of BN rats was dramatically reduced when coupled to 500 µg Protein A (Tables 1,2). The effect of Protein A on the reduction of spleen uptake of IC was seen at all time intervals after 1 hour (Figure 2). <sup>125</sup>I-p30 or <sup>125</sup>I-gp70 in normal serum or saline injected into normal BN rats i.v. exhibited no preferential sequestration in any body organ.

It is possible that Protein A reduced the uptake of IC by spleen by affecting antigen binding to its respective antibody. As shown in Table 3, Protein A has a slight but negligible effect on antigen binding. Protein A, therefore, will bind to IC and alter their distribution in the animal. It has been shown that Protein A is a T-cell mitogen (Nakaa

et al., 1980) so it could also alter the lymphoid tissues of animals. The mechanism of the effect of Protein A is currently under investigation.

Further studies will be required to determine whether the alteration by Protein A of the *in vivo* distribution of IC will benefit animals suffering from the pathological effects of immune complexes. We observed no gross toxic effects for rats treated with up to 1 mg Protein A i.v. Protein A has been reported to be non-toxic to rats and rabbits, but to exhibit toxic activity for guinea pigs and humans (Martin et al., 1967; Gustafson et al., 1968). Procedures that would reduce any toxic properties must be developed before Protein A could be administered to humans.

Table 1. Distribution of complexes of <sup>125</sup>I-p30-anti-p30 in tissues of rats.

Tissue	Treatment and Tissue/Blood ratio: <sup>a,b</sup>				
	Untreated	DES <sup>c</sup>	Trypan Blue <sup>d</sup>	Splenectomy <sup>e</sup>	PA <sup>f</sup>
Spleen	48.7±14.4	27.3±4.6	23.5±4.6	-	4.0±0.4
Liver	2.8±0.1	2.5±0.4	4.7±1.7	1.9±0.1	2.5±0.3
Lung	0.8±0.03	0.8±0.03	0.8±0.05	0.7±0.03	0.8±0.1
L. Nodes	1.9±0.4	0.7±0.1	1.2±0.2	1.1±.2	0.7±0.2
Thymus	0.6±0.1	0.6±0.1	0.6±0.05	0.5±0.1	N.D. <sup>g</sup>
Kidney	3.0±0.3	4.0±0.6	3.8±0.7	2.2±0.1	3.5±0.5

a) CPM <sup>125</sup>I-p30/100 mg tissue: CPM<sup>125</sup>I-p30/100 mg blood measured 24 hours after i.v. injection of 10 ng p30 contained in 50 µl BN anti-p30, average of 3-4 rats ± S.E.

b) If <sup>125</sup>I-p30 was injected with normal BN serum, ratios greater than 1.0 were not seen in any tissue, except kidney.

c) Diethylstilbestrol, 100 mg/kg dissolved in EtOH and injected i.p. 3 days before complexes (RES stimulant).

d) 50 mg i.p. at minus 24 hours and 10 mg at minus 4 hours before complexes (RES depressant).

e) 2 days prior to injection of complexes.

f) Protein A, 0.5 mg mixed with complexes (average of 7 rats).

g) Not done.

Table 2. Effect of protein A on distribution of immune complexes containing gp70.

Sample	Blood ratio at 24 hrs in: <sup>d</sup>			
	Spleen	Liver	Lung	L. Nodes
<sup>125</sup> I-gp70 in saline <sup>a</sup>	0.65±0	1.34±0.1	0.98±0.1	0.56±0.02
<sup>125</sup> I-gp70 in IC <sup>b</sup>	21.42±9.4	1.40±0.2	0.79±0.04	0.72±0.08
IC+PA <sup>c</sup>	1.30±0.1	0.86±0.1	0.76±0.02	0.80±0.08

a) 0.5 µg <sup>125</sup>I-gp70 of NaIV.

b) Immune complexes, 0.5 µg <sup>125</sup>I-gp70 + 200 µl hyperimmune BN anti-gp 70.

c) IC mixed with 1.0 mg protein A.

d) CPM/g tissue: CPM/g blood, average 3-4 rats ± S.E.

Effect of Protein A on Uptake of <sup>125</sup>I-Labelled Immune Complexes by Spleen

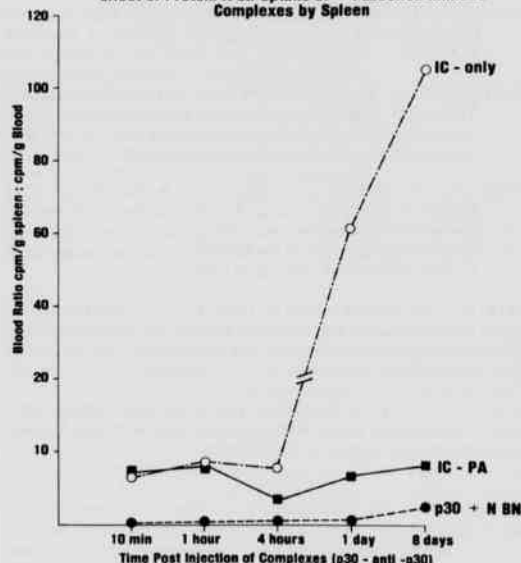


Figure 2. Effect of protein A on uptake of IC by spleen. Rats injected i.v. with IC (20 ng <sup>125</sup>I-p30 + 100 µl BN anti-p30) alone or mixed with 500 µg PA, or with <sup>125</sup>I-p30 in normal BN serum (NBN). Values plotted are blood ratios of spleen measured at 10 min., 1 hour, 4 hours, 1 day and 8 days post injection of IC, average of 4 animals/point ± S.E.

Table 3. Effect of protein A on antigen binding as determined by RIA.

Antibody <sup>a</sup>	Secondary Antibody <sup>c</sup>	%Prescription <sup>d</sup>
NBA	GAR	6.0
Anti-p30	GAR	66.0
NBN + PA <sup>b</sup>	GAR	15.8
Anti-p30 - p30+PA	GAR	60.7
NBN + PA	RAPA	2.0
Anti-p30+PA	RAPA	48.6

a) 5µl; NBN = normal BN; anti-p30 = BN anti-p30.

b) Protein A, 25µg incubated with antibody before addition of antigen (<sup>125</sup>I-p30).

c) 200 µl; GAR = Goat anti-rat globulin, RAPA = Rabbit anti-Protein A.

d) Percent of 1 ng <sup>125</sup>I-p30 precipitated in 30 minutes, average of 2 tests.

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