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Effect of Murine Tumor Sera on Adsorption of IgG-Sensitized Erythrocytes by Murine Sarcoma Tissue[1] (39672)

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Recent reports have shown that sera from animals bearing tumors (1) and from human patients with various types of neoplasms (2) contain immune complexes. Such immune complexes can bind specifically to tumor cells *in vitro* and block cytotoxicity of immune lymphocytes (1). Evidence also has been provided, by this laboratory and others, that immune complexes can bind "nonspecifically" in that sensitized sheep erythrocytes adsorb to tumor tissue via the Fc portion of the IgG molecule (3, 4). We have shown recently that immune complexes composed of soluble antigens and their corresponding antibodies can also bind "nonspecifically" to the Fc receptors of tumor cells, as well as to those on spleen cells (5). The binding of these complexes completely inhibited adsorption of antibody-sensitized erythrocytes by these tissues. We have considered that perhaps immune complexes composed of tumor antigen and antibody in sera from tumor-bearing animals could also bind "nonspecifically" to the Fc receptors of tumor cells. Accordingly, in the present study, sera from mice immunized with syngenic tumor were tested for their capacity to inhibit hemadsorption to Fc receptors of syngenic and allogenic tumor as well as spleen tissues.

Materials and methods. Animals. C3H/HeHa and C57BL/6 mice, 8-12 weeks old, from West Seneca Laboratories, West Seneca, NY, were used for all the experiments.

Tumor tissue. Methylcholanthrene-induced sarcomas, MCSa-22 and MCSa-3, were originally produced in this laboratory by subcutaneous injection of the carcinogen into C3H/HeHa and C57BL/6 mice, respectively. These tumors were maintained by transplantation in the strain of origin. Tumor tissue was usually collected within 2-3 weeks after transplantation. If the tissue was not used immediately, it was stored at −70°. Histopathological examination of paraffin-embedded sections, as well as of cryostat sections, were performed for each tumor specimen tested. Cryostat sections for histopathological examination were taken randomly from sections cut for each hemadsorption experiment. Only neoplastic tissue free from necrosis and inflammatory cell infiltration was used in this study.

Immune sera. C3H/HeHa and C57BL/6 mice were immunized with syngenic tumors by subcutaneous implantation with a trocar of a 3 × 30 mm plug of minced tumor tissue. In 2-3 weeks, when the tumor attained 2-2.5 cm in diameter, it was excised. Four days later, the mice were inoculated again with the same dose of minced tumor tissue. Again, the tumors were excised in 2-3 weeks, and 4 days later, the entire group was inoculated for a third time. The mice were bled 3-5 days after the third inoculation of tumor.

All sera were placed at 4° and were tested for inhibition of adsorption of sensitized sheep erythrocytes within 24 hr. Some of these sera were retested after storage at −70°. However, repeated freezing and thawing of these sera was avoided.

Hemadsorption and inhibition of hemadsorption. Hemadsorption by cryostat-cut tissue sections was performed as previously described (3, 5). Briefly, 10 μm thick tissue sections were placed on a coverslip and dried at room temperature. Wells of microculture slides were filled with a suspension of sheep erythrocytes sensitized by a selected subagglutinating dilution of a rabbit anti-sheep erythrocyte serum. The wells were sealed with the coverslips in such a way that the tissue section was submerged in the suspension of sensitized erythrocytes. The

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preparations were incubated, coverslip down, for 1 hr at room temperature. Then they were turned, coverslip up, and left at this position at room temperature to allow unattached erythrocytes to fall down from the tissue. The sections were then examined microscopically at low magnification.

Adherence of sensitized sheep erthrocytes to the sections was recorded as a positive hemadsorption test. If a tissue section was completely covered by sensitized sheep erythrocytes, the test was graded as 4+. A reaction was considered 2+ if approximately half the tissue was covered by erythrocytes. Sparsely attached erythrocytes on the tissues was considered as a 1+ reaction. Absence of erythrocytes from the tissue was recorded as a negative result.

Inhibition studies were performed by incubating tissue sections in a moist chamber at room temperature for 20 min with various sera or with phosphate buffered saline, pH 7.2 (PBS). Subsequently, the tissue sections were washed three times in PBS and the hemadsorption test was performed. In order to avoid experimental variation, all data presented in each of the tables were obtained on the same day.

Results. Sera from 72 C57BL/6 and 68 C3H/HeHa mice immunized with the syngenic tumors were tested for inhibition of hemadsorption. Virtually all sera tested gave inhibition. An example of these results is shown in Table I, in which the immune serum inhibited hemadsorption up to a dilution of 1:40, whereas normal serum did not inhibit even at a dilution of 1:10. It should be noted that inhibition was also obtained with immune serum heated at 56° for 30 min. None of 72 sera from normal C57BL/6 and C3H/HeHa mice gave inhibition.

TABLE I. ADSORPTION OF SENSITIZED SHEEP ERYTHROCYTES BY C57BL TUMOR TISSUE. INHIBITION BY SERUM FROM A NORMAL C57BL MOUSE AND A C57BL MOUSE IMMUNIZED WITH SYNGENIC TUMOR.

	Serum from:			
Dilution of serum 1 to:	Normal mouse	Immunized mouse	Heat inactivated serum from immunized mouse	Saline
10	2+	–	–	
20	3+	–	–	3+
40	3+	–	–	

The specificity of inhibition of the hemadsorption with sera from mice immunized with syngenic tumors was also investigated. An example of such an experiment is given in Table II. Immune sera from C3H mice as well as those of C57BL mice inhibited hemadsorption by both C3H and C57BL tumors. As seen in Table III, similar inhibi-

TABLE II. ADSORPTION OF SENSITIZED SHEEP ERYTHROCYTES BY TUMOR TISSUE FROM C3H AND C57BL MICE. INHIBITION BY SERA FROM NORMAL C3H AND C57BL MICE AND C3H AND C57BL MICE IMMUNIZED WITH SYNGENIC TUMOR.

		Hemadsorption by tumor from	
Sera from	Dilution 1 to:	C3H	C57BL
Immunized C3H	10	–	–
	20	–	–
	40	–	–
Normal C3H	10	3+	3+
	20	4+	4+
	40	4+	4+
Immunized C57BL	10	–	–
	20	–	–
	40	–	1+
Normal C57BL	10	3+	3+
	20	4+	4+
	40	4+	4+
Saline		4+	4+

TABLE III. ADSORPTION OF SENSITIZED SHEEP ERYTHROCYTES BY NORMAL SPLEEN TISSUE FROM C3H AND C57BL MICE. INHIBITION BY SERA FROM NORMAL C3H AND C57BL MICE AND C3H AND C57BL MICE IMMUNIZED WITH SYNGENIC TUMOR.

		Hemadsorption by spleen from	
Sera from	Dilution 1 to:	C3H	C57BL
Immunized C3H	10	–	–
	20	–	–
	40	1+	–
Normal C3H	10	3+	3+
	20	3+	3+
	40	3+	3+
Immunized C57BL	10	–	–
	20	–	–
	40	1+	1+
Normal C57BL	10	2+	2+
	20	3+	3+
	40	3+	3+
Saline		3+	3+

tion results were obtained when normal spleen tissue from C3H and C57BL mice were used in place of tumor tissue.

Discussion. Previous work in this laboratory (3) showed that murine methylcholanthrene-induced sarcomas (MCSa) adsorb sheep erthrocytes sensitized by IgG but not unsensitized erythrocytes or erythrocytes sensitized by IgM antibodies. All these results were fully confirmed at the beginning of the present study.

This study demonstrated that sera from mice immunized with syngenic MCSa inhibited the attachment of sensitized sheep erythrocytes to MCSa. In contrast, sera from normal animals did not inhibit hemadsorption. The inhibition of hemadsorption by the immune sera could have been due to binding of tumor-specific antibody to the corresponding antigen on the tumor cell, which might have been identical to the Fc receptor or in such close proximity to them as to render the receptor inaccessible. Alternatively, immune complexes composed of tumor antigen and its corresponding antibody could bind "nonspecifically" to the Fc receptors of the tumor cells. The latter possibility is most likely since we have shown in a previous study (5) that BSA rabbit-anti-BSA complexes may bind to Fc receptors of murine tumors and, consequently, inhibit hemadsorption. Furthermore, in this study, sera of tumor-bearing mice inhibited hemadsorption by allogenic, as well as syngenic tumors. In addition, inhibition of hemadsorption was demonstrated with these sera on normal spleen tissue, which obviously do not bear tumor-specific antigens.

Preliminary studies with Raji cell technique (6) (not reported in the results) were consistent with the described results in that of 11 tumor sera tested, all contained immune complexes detectable by this technique.

It could be argued that complement receptors, as well as Fc receptors, could be involved in the hemadsorption phenomenon, and its inhibition by immune murine sera, since tumor cells are known to bear complement receptors (7). Participation of complement receptors was excluded, however, by the finding that inhibition of hemadsorption was not altered by heat-activation of the inhibiting sera.

Summary. Previous work in this laboratory has shown that the adsorption of IgG antibody-sensitized erythrocytes by tumor and normal spleen tissues can be inhibited by immune complexes and heat-aggregated IgG. The present study showed that sera from mice immunized with syngenic tumor cells inhibited adsorption of sensitized erythrocytes by tumor and spleen tissues. These sera inhibited also hemadsorption by allogenic tumor tissue and, significantly, by syngenic as well as allogenic spleen tissue.

These results support the contention that sera from tumor-bearing animals contain immune complexes which block the Fc receptors of tumor and spleen cells indiscriminately.

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