ANTIBODY-MEDIATED SUPPRESSION OF GRAFTED LYMPHOMA CELLS

II. Participation of Macrophages

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In the presence of humoral antibody directed against grafts of various tumors, hosts syngeneic with the tumors may reject the grafts (1). We have reported that an intact complement system is not required for antibody-mediated suppression of certain syngeneic murine lymphoma grafts. A radiosensitive host factor(s), however, is (are) involved in this reaction as evidenced by the failure of irradiated hosts to suppress grafts that had been admixed with specific alloantibody (2).

The present report concerns the delineation of a host factor(s) involved in antibody-mediated suppression of grafts of the C3H lymphoma 6C3HED in C3HeB/J mice. Various C3HeB normal cell populations and sera were assayed for their capacity to restore suppressive ability to irradiated mice.

Materials and Methods

Details on the mice of inbred strains C3HeB/FJ and C57BL/6J (abbreviated B6),[1] C3H lymphoma 6C3HED, B6 anti-6C3HED serum, and whole body X-irradiation are found in reference 2.

Normal Donor Cells (NDC).—Isolated thymus, spleen, or lymph nodes from C3HeB mice were transferred to a Duall glass homogenizer (Kontes Glass Co., Vineland, N. J.) which contained Hanks' balanced salt solution plus 0.1% glucose and 0.01% bovine serum albumin (HBG) at room temperature in a volume more than ten times that of the organs. The organs

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[1] *Abbreviations used in this paper*: B6, C57BL/6J; HBG, Hanks' balanced salt solution plus 0.1% glucose and 0.01% bovine serum albumin; HBGH, HBG containing 5 units of heparin/ml; NDC, normal donor cells.

were disrupted by one or two rotating thrusts of the plunger. The free cells were decanted to a centrifuge tube and were washed once by centrifugation in HBG. The pellet was dispersed in fresh HBG and 3 min were allowed for clumps to settle. The top two-thirds of the suspension was aspirated and its cell concentration was adjusted in HBG to the desired value. Marrow was flushed out from femurs with HBG containing 5 units of heparin/ml (HBGH) and was dispersed in a homogenizer. After two washes by centrifugation in HBG the cell pellet was dispersed in HBG.

For cells from the peritoneal cavity, 5 ml of HBGH were injected intraperitoneally in a killed mouse. After the mouse was shaken gently, the fluid was aspirated and its cells were washed twice in HBG. Six categories of donors were used: (*a*) normal mice; (*b*) normal mice given 3 ml each of beef infusion broth intraperitoneally 2 days before cell harvest; (*c*) normal mice given 3 ml each of beef infusion broth intraperitoneally 4 days before cell harvest; (*d*) mice given 3 ml each of 2.95% thioglycollate (Difco Laboratories, Inc., Detroit, Mich.) intraperitoneally 6 days before cell harvest; (*e*) mice treated as in category (*c*) except that they were given 500 R whole body irradiation 20 hr before cell harvest; (*f*) as in category (*d*) except mice were given 500 R whole body irradiation 20 hr before cell harvest.

Cultured Macrophages.—The method described in reference 3 was used to culture the cells from category (*b*) mentioned above. Cell viability was 87% by dye exclusion.

Experimental Tumor Grafting.—Tumor cells were harvested from C3H mice as described (2) and the cell count was adjusted to 8×10^6/ml HBG. For injection, aliquots of the tumor cell suspension were mixed in equal volume with normal serum or antiserum that had been diluted 1/2.5 with HBG, the mixtures being kept in crushed ice. After 15 min and with frequent shaking, two parts of HBG alone or HBG containing NDC were mixed with one part of each tumor cell suspension. Immediately thereafter 0.05 ml of the suspension (i.e., 10^5 tumor cells plus varying numbers of NDC) was inoculated in one calf muscle of each mouse. Graft growth was followed by daily caliper measurement, as described (2).

RESULTS

Sensitivity of Mice to X-Irradiation.—As reported previously (2) tumor growth was not inhibited in C3HeB mice given whole body irradiation and inoculated 1 day later with 6C3HED cells admixed with anti-graft alloantibody. This was in contrast to successful suppression in nonirradiated mice. At 400 R loss of suppressive capacity was inconsistent but at 500 R or above all mice were incapacitated.

For a kinetic study of loss and recovery of suppressive ability, groups of mice exposed to 500 R were challenged at various time intervals thereafter with tumor alone or tumor admixed with antibody ("sensitized" tumor). Loss of capacity was most prevalent in mice inoculated 1 or 2 days after irradiation (Fig. 1 B); there was a marked loss of ability with an interval as short as 3 hr. By 3 days recovery was complete. In subsequent experiments, therefore, mice were challenged with tumor 1 day after irradiation.

Restoration of Activity with NDC.—Tumor cells, sensitized or not, were mixed with NDC and inoculated into irradiated hosts. Growth of the tumors was followed by daily measurement. Tumor size on day 11 after inoculation is tabulated in Table I. Only peritoneal cells restored suppressive capacity. These cells, however, varied in their "curative" potential. In three additional experiments peritoneal cells from untreated mice gave two partial successes and one

failure. In contrast, peritoneal exudate cells induced either by beef broth or thioglycollate consistently restored suppressive capacity. In four separate experiments spleen, thymus, lymph node, or marrow cells, separately or in

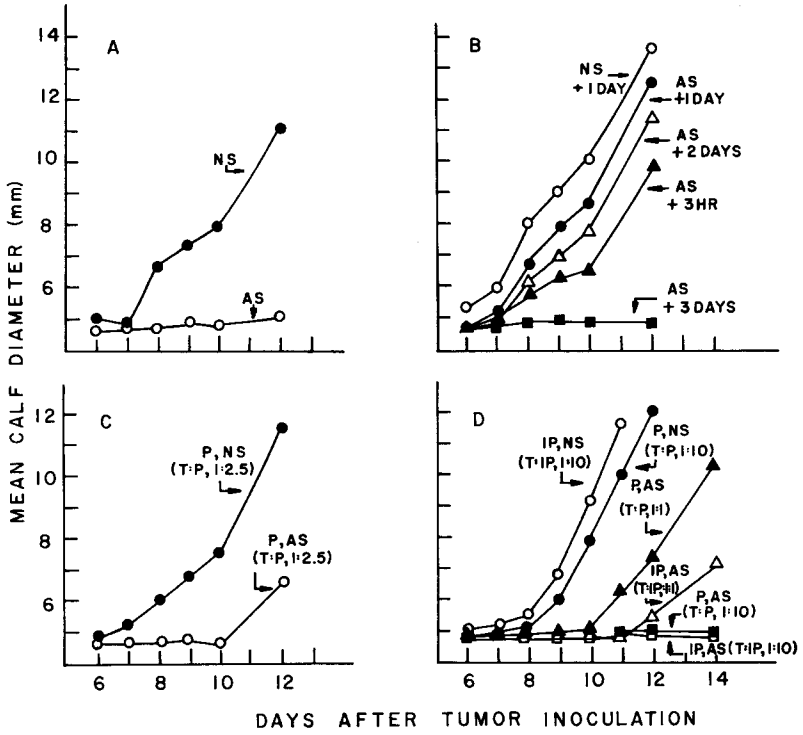

FIG. 1. Growth of 6C3HED grafts in calf muscles of C3HeB females (five per group). Abbreviations: AS, tumor inoculum admixed with anti-tumor alloantiserum; NS, tumor inoculum admixed with normal serum; P, peritoneal cells from normal C3HeB mice; IP, peritoneal cells from irradiated donors; T, tumor cells. (A) Graft growth in nonirradiated hosts. (B) Kinetics of host recovery from irradiation-induced incapacitation for graft suppression. Curves show growth rates for grafts inoculated at 3 hr and at various days after irradiation. (C) Graft growth in mice given whole body irradiation 24 hr before tumor inoculation. Tumor inocula admixed with cultured peritoneal macrophages. Ratio of tumor cells to admixed peritoneal cells given in parentheses. (D) Graft growth in mice given whole body irradiation 24 hr before tumor inoculation. Tumor inocula admixed with thioglycollate induced peritoneal exudate cells from normal or irradiated donors. Ratio of tumor cells to admixed peritoneal cells given in parentheses.

combination, were ineffective when admixed with the tumor inoculum, as was normal serum injected intraperitoneally shortly before tumor grafting.

Cultured Macrophages as Effectors.—To identify the peritoneal exudate cells (induced by beef broth) that restore suppressive capacity, macrophages were isolated by cell culture. The cultures contained 99.5% macrophages and 0.5%

lymphocytes, in contrast to the 93% macrophages and 7% lymphocytes of uncultured cells. The cultured macrophages did restore suppressive capability in irradiated mice (Fig. 1 C).

Restoration with Cells from Irradiated Donors.—Mice receiving thioglycollate intraperitoneally were given 500 R whole body irradiation 5 days later. The

TABLE I

Peritoneal Cells Admixed with the Tumor-Antibody Inoculum Restore the Ability of Irradiated Mice to Suppress Tumor Isografts

Admixed normal donor cells*		Host irradiated (500 R)	Antiserum mixed with tumor*	Tumor size on day 11‡	
Type	No. per inoculum ($\times 10^5$)			No. of mice with tumor‡	Mean calf diameter ± SE
					mm
None	0	−	−	5	9.7 ± 0.45
None	0	−	+	0	4.8 ± 0.04
None	0	+	−	5	10.8 ± 0.69
None	0	+	+	5	10.8 ± 0.57
Peritoneal	9	+	−	5	12.5 ± 0.36
Peritoneal	9	+	+	0	5.1 ± 0.02
PC-BIB	4	+	−	4	10.0 ± 1.14
PC-BIB	4	+	+	1	5.3 ± 0.48
PC-TGL	10	+	−	5	10.1 ± 0.46
PC-TGL	10	+	+	0	4.9 ± 0.08
Spleen	10	+	+	5	11.0 ± 0.34
Lymph node	10	+	+	5	12.0 ± 0.37
Thymus	10	+	+	5	12.7 ± 0.57
Bone marrow	10	+	+	5	10.2 ± 0.40
Thymus, bone marrow	10 each	+	+	5	11.6 ± 0.40
Spleen, thymus, bone marrow, lymph node, normal serum	2.5 of each cell type, 0.4 ml serum i.p.	+	+	5	10.7 ± 0.48

* PC-BIB, peritoneal cells from exudate induced by beef infusion broth; PC-TGL, peritoneal cells from exudate induced by thioglycollate; cells admixed with 10^5 tumor cells per inoculum. Antiserum diluted 1/2.5 in HBG.

‡ Five females per group.

exudate was harvested 20 hr after irradiation; it contained 99.5% macrophages and 0.5% lymphocytes. Similarly induced exudate of nonirradiated mice contained 91% macrophages and 9% lymphocytes. Both groups of cells were about equally tumor suppressive in irradiated hosts when admixed with the tumor-antibody inoculum (Fig. 1 D).

Exudate cells harvested 4 days after an intraperitoneal injection of beef broth were also checked for radiosensitivity. A group of donors was given 500 R whole body irradiation 20 hr before cell harvest. Cells from irradiated

mice (95% macrophages, 5% lymphocytes) were as competent as those of non-irradiated donors (81% macrophages, 18% lymphocytes, 1% polymorphonuclear leukocytes) in effecting tumor suppression in irradiated hosts.

DISCUSSION

We have found that antibody was not suppressive for 6C3HED grafts in irradiated C3HeB mice despite the presence of normal or elevated levels of complement (2). The present study demonstrates that various types of peritoneal cells or highly purified macrophages admixed with sensitized tumor cells restore suppressive capacity of irradiated mice. Apparently, macrophages participate in antibody-mediated graft suppression.

Tsoi and Weiser (4) reported that allogeneic grafts of the strain A tumor sacroma I in irradiated C57BL/Ks mice could be suppressed by starch-induced C57BL/Ks peritoneal exudate cells in the presence of C57BL/Ks anti-sarcoma I serum. Peritoneal cells from immune mice were more effective. The exudate cells were not further characterized and other cell types were not examined. Other investigators have shown the participation of macrophages (5) or normal lymphocytes (reviewed in 6) in vitro in antibody-mediated target cell destruction.

Though only macrophages in our experiments restored suppressive capacity, a requisite for other cell types is not precluded. Should such cells be radioresistant, an effective number could have survived radiation in the tumor host. Moreover, since our adoptive cell transfer was in the tumor inoculum site, we have not entertained the question of how macrophage precursors are recruited in the normal animal and how they differentiate and arrive at the graft site. Other cell types may participate in these events.

It would seem paradoxical that irradiated mice can be "restored" by peritoneal cells from irradiated donors, thus indicating that these cells are radioresistant. The incapacity of irradiated mice may be attributed to: (*a*) radiosensitive macrophage precursors; or (*b*) an as yet unidentified radiosensitive factor(s) which is needed for optimal macrophage function; or (*c*) both categories (*a*) and (*b*) may be operative. Studies in progress are directed toward "reconstituting" irradiated animals systemically rather than locally to further define the host effectors in graft rejection.

Our conclusion that an intact complement system is not required for graft suppression by antibody and that macrophages are involved in this reaction is based on experience with a limited number of murine tumors. Whether this is true also for other murine tumors or for species other than the mouse or what general significance various host factors may have in a graft situation remains to be determined.

SUMMARY

Specific alloantibody admixed with a grafted murine lymphoma is suppressive of the graft in mice of the inbred strain native to the tumor. Suppressive

capacity of the host is obviated in mice given 500 R whole body irradiation before tumor inoculation but is restored when normal peritoneal macrophages are admixed with the tumor-antibody inoculum. Other normal cell types admixed with the tumor-antibody inoculum are not effective in restoring suppressive capacity.

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