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Immunology

Influence of dendritic cells on tumor growth

(antigen-presenting cells/tumor immunity/vitamin A acetate/tolerance)

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ABSTRACT **Dendritic cells (DC) exposed to antigen are potent initiators of immune responses, and the numbers of DC and the dose of antigen control the level of response. The influence of these variables was tested on the growth of mouse sarcoma cells *in vivo*. When normal syngeneic DC (100,000) were given to mice with palpable tumors, tumor regression or delay in tumor growth was obtained. DC exposed to increasing doses of tumor extract *in vitro* before administration had progressively less effect. DC exposed to antigen delayed tumor growth significantly only when given on the same day as 500 tumor cells. The studies suggested that low doses of antigen on DC elicit immune responses and that high doses block them. The numbers of antigen-presenting cells and the dose of antigen modulate the degree of immunity to mouse sarcoma *in vivo*.**

Dendritic cells (DC) stimulate immune responses to intrinsic membrane antigens or to extrinsic antigens attached to their cell surfaces (1–7, 9, 14, 15). They possess high levels of Ia antigens and initiate mixed leukocyte reactions (1–7). In mice, as few as 10,000 cells initiate host versus graft responses *in vivo* (9). When exposed to picryl chloride, small numbers of DC from mice trigger primary immune responses *in vitro* and *in vivo* (3). DC also may elicit responses to autoantigens, since 80,000 DC from rats with the autoimmune disease experimental allergic encephalomyelitis injected into normal recipients initiated some symptoms of disease (9). The levels of responses are altered by changing the numbers of DC and the dose of antigen. Thus, DC with high doses of antigen inhibit proliferative response *in vitro* (2), and high numbers of semi-allogeneic DC result in reduced host versus graft activity *in vivo* (9). The present experiments investigated whether manipulation of the numbers of DC and the amount of tumor antigen to which they were exposed could modulate tumor immunity in mice.

MATERIALS AND METHODS

Cell suspensions of spleen from normal CBA mice were cultured at 5×10^6 cells per ml of medium (RPMI 1640 medium, Dutch modification with 100 units of penicillin and 100 μg of streptomycin per ml, 10 μM 2-mercaptoethanol, and 10% fetal calf serum; Flow Laboratories) for 24 hr in tissue culture flasks (Nunc). This cell suspension was layered onto a gradient of metrizamide (Nyegaard; 14.5 g and 100 ml of medium) and was centrifuged at $600 \times g$ for 10 min. The interface cells were morphologically mainly DC (>70%), had high levels of Ia antigen, and showed little or no staining for nonspecific esterase. They contained <10% lymphocytes and <5% phagocytic cells that formed rosettes with erythrocytes coated with antibody (9).

Sarcoma cells from a methylcholanthrene-induced tumor of a CBA mouse (McSa-1) were cloned and then stored in liquid nitrogen (8). Samples were thawed and grown in culture for 2 days before injection of 500 cells subcutaneously into the nuchal region of CBA mice. Palpable tumors were generally found between 2 and 3 weeks later (8). To prepare tumor extract, we aseptically removed the tumor, discarding necrotic tissue; the tumor was washed with balanced salt solution, weighed, and, after addition of an equal amount (wt/vol) of balanced salt solution on ice, homogenized. The homogenate was sieved to remove any remaining tissue fragments, shell-frozen, freeze-dried under reduced pressure, and stored at −30°C over silica gel.

DC isolated from spleens were incubated at 37°C for 2 hr in medium or in medium containing 1, 10, or 100 μg of freeze-dried tumor extract per ml. The DC were washed, resuspended in fresh medium, and injected intravenously into mice.

RESULTS

Effect of DC Injected into Mice with Palpable Tumors. The effect of intravenous injection of 100,000 DC from normal CBA spleens (9) was tested on the growth of mouse sarcoma (8). Animals receiving subcutaneous injections of 500 tumor cells developed tumors and died, except for occasional animals exhibiting 'spontaneous' regression of tumor (e.g., Fig. 1). Half of the animals with palpable tumors (2–3 weeks after injection of tumor cells) given normal DC showed tumor regression [Fig. 1; $P = 0.03$, compared with animals without DC (10)]. When DC were treated with freeze-dried tumor material before injection (0–100 μg), the regression was reduced in relation to the increase in antigen dose (Fig. 1). The significance of this linear trend was $P = 0.02$ (10). Mice fed on a diet rich in vitamin A acetate had more splenic DC (11). DC from vitamin A acetate-fed mice also inhibited tumor growth (Fig. 2: $P = 0.03$). Tumor regression was again reduced with DC treated with increasing doses of antigen (Fig. 2: P value for the linear trend = 0.007). In three experiments direct comparison was made between DC from mice fed on vitamin A acetate and those on conventional diets. No reproducible difference in the effect on tumor growth using DC from these two feeding groups was seen on treatment with 100,000 DC.

Effect of DC Given at the Same Time as Tumor Cells. DC (100,000) were given to normal mice on the same day as 500 tumor cells. A small but significant delay in tumor growth was obtained only when the DC were treated with tumor antigen before injection, as demonstrated in Fig. 3. When animals were given DC treated with antigen at 100 μg/ml, the delay in appearance of palpable tumor was significant ($P = 0.008$). In contrast with results in mice having established tumors, treated DC increased in effectiveness with increase in the

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Abbreviation: DC, dendritic cells.

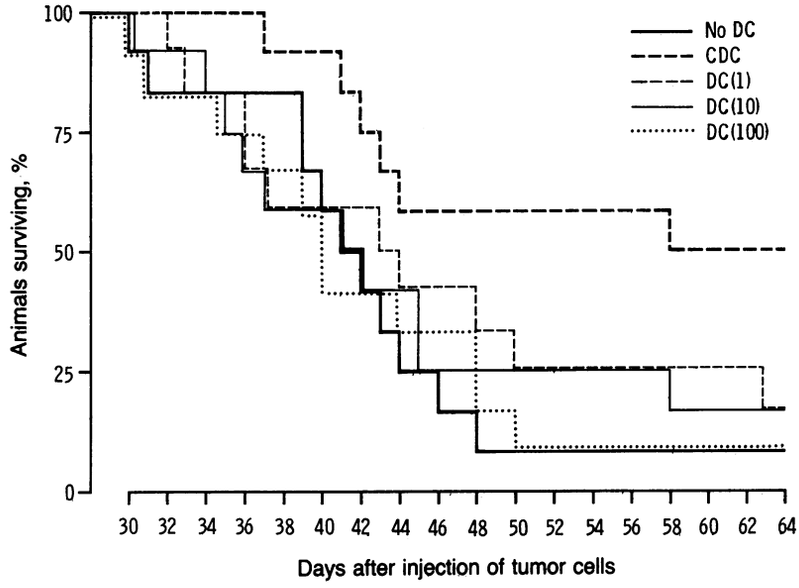

FIG. 1. Effect of intravenous injection of dendritic cells (DC) into mice with palpable tumors. Mice with tumors (18 days after injection of 500 tumor cells) received no DC or received intravenous injections of 100,000 normal DC (CDC) or 100,000 DC exposed to 1, 10, or 100 μg of tumor extract per ml as indicated (12 animals per group). Animals surviving at different times after injection of tumor cells are shown. Mice surviving were free of tumor.

amount of antigen (P = 0.0008 for the linear trend). There were no significant changes in the rates of death in the experiment shown in Fig. 3 (P = 0.14), but marginally significant delay in death was observed in another experiment (P = 0.05; data not shown).

DISCUSSION

These experiments showed that tumor antigen on DC was required to initiate immunity but also could inhibit immune responses to tumor. This apparent contradiction was resolved in two preliminary experiments, which showed that there was only a narrow dosage range for tumor antigen on DC giving initiation of immunity. In the first experiment, the treated DC were given to mice 2 weeks after the tumor cells but before the mice had palpable tumors. In the second experiment, treated DC were given at the same time as tumor cells, and a different batch of freeze-dried tumor antigen was used. In both experiments inhibition of tumor growth only occurred when the DC were treated with antigen at 10 μg/ml but not with cells exposed to tumor antigen at 1 or 100 μg/ml. The effective range of antigen provided by tumor cells *in vivo* may also be narrow. In animals receiving 500 tumor cells, the initial antigen dose is presumably low, and pulsing of DC with some intrinsic antigen may be required to reach a stimulatory level. By contrast, mice with palpable tumors presumably have high levels of antigen provided by the tumor. DC may acquire this antigen from the tumor cells so that pulsing DC with extrinsic antigen is inhibitory.

DC and Langerhans cells (which are precursors for some DC in lymph nodes draining the skin) can pick up antigen on their cell surfaces and initiate immune responses *in vivo* (1, 2, 12). Since identification and isolation of tumor-specific antigen is difficult, we used crude, freeze-dried tumor extract as a source of tumor antigen in this study. To obtain antigenic material, we exposed DC to this extract *in vitro* and relied on

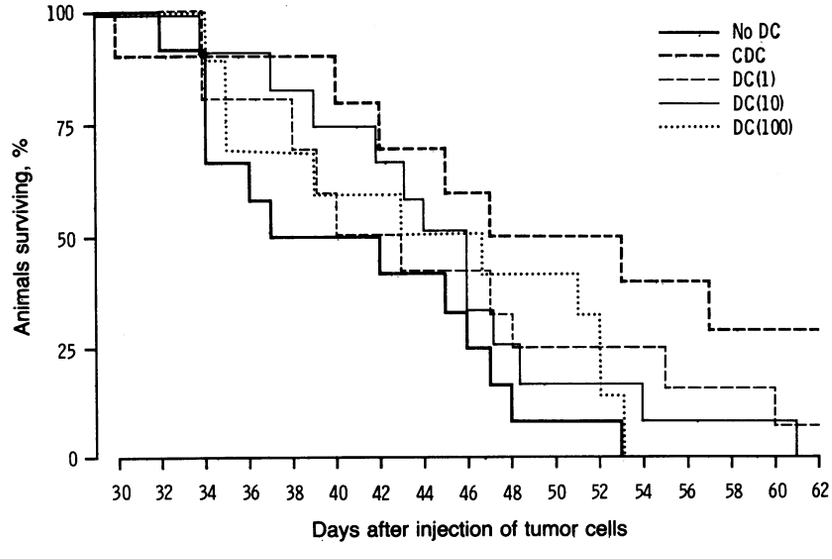

FIG. 2. Same as Fig. 1 except that DC were isolated from the spleens of mice fed a diet rich in vitamin A acetate (8). There were 10–12 mice per treatment group.

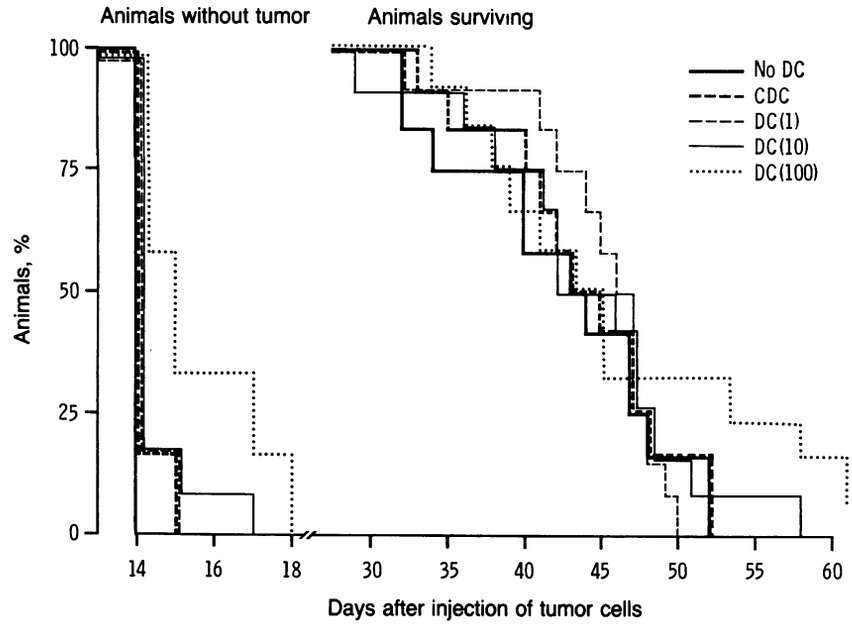

FIG. 3. Details are as for Fig. 2 except that mice received intravenous injections of DC on the same day as the subcutaneous injection of 500 tumor cells. Animals developing palpable tumors (*Left*) and the percentage surviving (*Right*) are shown at different times after injection of the tumor cells and the DC.

the innate capacity of DC to pick up antigen, but the specificity of this material is unknown. Lymphoid cells from mice with tumors can initiate immunity in normal recipients (13). One explanation for this effect could be the presence of DC that have acquired tumor antigens in the lymphoid tissue of tumor-bearing mice.

In conclusion, this work demonstrates that DC can pick up tumor antigen and modulate tumor immunity. Low doses of antigen on DC can initiate immunity to tumors, and high doses of antigen on DC block tumor immunity. The findings support the possibility that immunological unresponsiveness in tumor-bearing animals can result from high doses of tumor antigen (12).

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