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Natural Resistance Mechanisms May Play a Role in Protection Against Chemical Carcinogenesis

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Summary. *Mice deprived of B lymphocytes by the chronic administration of anti-IgM antibodies have been shown to possess a heightened natural resistance (NR) to micro-organisms, to parental bone marrow, and to natural killer (NK)-sensitive tumors in vitro and in vivo. Experiments described in this communication indicate that the latent period of primary tumors induced by the injection of methylcholanthrene (MC) is also prolonged in these mice. This observation suggests that NR mechanisms may provide protection against primary chemically induced tumors.*

Introduction

We have previously shown that mice deprived of B lymphocytes by chronic administration of anti-IgM antibodies possess a heightened resistance to transplants of a methylcholanthrene (MC)-induced tumor of recent origin [3]. Subsequent experiments have disclosed that one consequence of the depletion of B lymphocytes is a significant increase in natural killer (NK) activity [4], another being a marked augmentation of other parameters of natural resistance (NR), such as hybrid resistance to parental bone marrow and resistance to infection by intra- and extracellular parasites [5]. These observations, along with a lack of demonstrable T cell-mediated immunity against the MC-induced tumor, led us to believe that the augmented NR was responsible for the heightened antitumor activity of the anti-IgM-treated mice. The purpose of the experiments to be described was to determine whether the heightened NR of B lymphocyte-deprived mice could be shown to affect the incidence of primary tumors elicited by the injection of 3-MC.

Materials and Methods

Animals. (C57BL/6×C3H)F1 mice purchased from BioBreeding Laboratories Ottawa, Ontario were used throughout this investigation. Carcinogenesis was initiated by the injection of 3-MC in 7-week-old male mice. Assays of NK activity were performed in animals of both sexes, at the ages indicated.

Anti-IgM-Mediated Suppression. The procedure for the production of rabbit anti-IgM serum and suppression of neonatal animals has been previously described [10]. Briefly, serum pools from 50–90 rabbits immunized with IgM derived from Balb/c plasmacytoma MOPC 104-E were precipitated twice with ammonium sulfate, and the final product obtained at 33% saturation was dialyzed, centrifuged at 1000,000 *g*, and frozen in small aliquots. A normal rabbit serum pool (NRG) purchased from Pel Freez Biologicals, Inc. (Rogers, AR, USA) was processed in an identical manner. Before use, antibodies to mouse red cells were removed from both preparations by absorption with rat and mouse erythrocytes fixed with 0.5% glutaraldehyde. For suppression of neonatal mice, 5–10 mg of the serum preparation in a volume of 0.05–0.1 ml was injected within the first 2 days of life and three times a week thereafter for the duration of the experiment.

Mice treated with anti-IgM from birth by the above procedure have been shown to be deprived of B lymphocytes and of B lymphocyte functions. Analyses carried out on mice up to the age of 20 weeks or longer included tests for surface Ig-bearing cells by radioautography [20, 21], and by the fluorescence-activated cell sorter (P. De Baetselier and J. Gordon, unpublished observations), for serum IgM by immunodiffusion and by a radioimmunoassay (K. Abikar and J. Gordon, unpublished work), and for the production of specific antibody by immunization with sheep erythrocytes (K. Abikar and J. Gordon, unpublished work). Other tests carried out on mice up to 31 weeks of age included activation of cells in vitro with LPS and immunization with a variety of antigens [10]. Mice used throughout these studies were defined as B cell-deprived according to the above criteria. Some of these mice possessed a variable, low level of serum IgG, which probably represents secretion from long-lived cells and does not have any bearing on the immunocompetence of these animals (K. Abikar and J. Gordon, unpublished work).

The immunosuppressed status of the mice was routinely confirmed at 5–6 weeks of age by assay of serum IgM and by the detection of an excess of circulating rabbit anti-IgM antibodies. Throughout this paper mice treated with anti-IgM in this manner will be referred to as suppressed, anti-IgM-treated, or B cell-deprived, interchangeably.

Mice treated from birth with NRG were normally used as controls. They are referred to as NRG-treated or controls. In some experiments non-treated mice were also employed, and they are referred to as normal mice.

Tumor Induction. 3-Methylcholanthrene was used to induce tumors according to the method of Klein et al. [15].

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Abbreviations used in this paper: MC, methylcholanthrene; NR, natural resistance; NRG, normal rabbit serum pool; NK, natural killer cell

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Seven-week-old male mice weighing 15–20 g received 0.5 mg MC dissolved in 0.1 ml trioctanoin oil by IM injection in the right hind leg.

Tumors were measured three times weekly with calipers. Mean tumor diameters were calculated from measurements in two planes at right angles and were registered positive when they exceeded 0.3 cm.

Assays of NK Activity. Assays were carried out with a tissue culture line of the YAC lymphoma cells (YAC-l) used as target. These cells were labelled by incubating 5×10^6 cells in 0.2 ml 100 μCi/ml $Na_2{}^{51}CrO_4$ solution for 1 hour, followed by three washes. Labelled target cells (1×10^4) were mixed with different numbers of effector cells in a total volume of 0.2 ml in microtiter plates and incubated for 4–5 hours with rocking at 7 cycles/min. Each combination was assayed in triplicate and the cytotoxic activity was expressed as percentage specific release according to the formula:

$$\frac{\text{CPM experimental} - \text{CPM spontaneous release}}{\text{Total CPM} - \text{spontaneous release}} \times 100 .$$

Each assay was carried out on pooled spleen cells from three or four mice.

Statistical Analysis. Tumor incidence was computed for each group of mice at different time intervals following administration of the carcinogen. The data are presented as the cumulative probability of mice remaining tumor-free at each time interval, calculated from the tumor incidence by the life table approach [7] according to the formula:

$$qx = \frac{dx}{ox - wx/2}$$

where dx denotes the number of tumors which appeared during the given time interval, ox the number of animals under observation, and wx the number of withdrawals, i.e., the number of mice that died during the same interval without tumors. The statistical significance of the results was evaluated by the life table test. Student's *t*-test was used for the analysis of results obtained in the NK assays.

Results

The NK activity of B lymphocyte-deprived and of normal control mice is compared in Fig. 1. As can be seen, the activity of the former group is augmented, and it is sustained in older animals. This increased NK activity shown here against YAC-1 target cells was also demonstrable against several MC-induced sarcomas, a spontaneous mammary carcinoma, and a melanoma [4].

Two experiments were carried out to study the incidence of primary tumors in anti-IgM-treated mice injected with MC. In the first, only 12 anti-IgM-treated and eight NRG-treated animals were used. Although the difference in tumor incidence between the two groups was not statistically significant, the proportion of animals with tumors was lower among the suppressed animals than among the controls throughout 100 days of observation. In the second experiment, in which 30 normal, 22 B lymphocyte-deprived and 20 NRG-treated control animals were used, the tumor incidence among the anti-IgM-treated animals was significantly lower than in the two control groups (which did not differ from each other) up to 94 days after the initiation of the experiment, when all mice developed tumors. Typically, tumor incidence 74 days after injection of the carcinogen was three of 20 (14%) for the anti-IgM-treated group, seven of 19 (37%) for the NRG-treated mice, and 12 of 30 (40%) for the untreated group; at 84 days the incidences in the three groups were five of 13 (38%), 13 of 18 (72%), and 22 of 30 (73%), respectively. These data, computed as the cumulative probability of mice remaining tumor-free, are shown in Fig. 2. The difference between the anti-IgM-treated group and the two control groups was significant at every time interval tested ($P < -0.01$) up to 94 days after the injection of the carcinogen.

Discussion

Despite the clear demonstration that among all experimental tumors, those induced by chemicals are the most antigenic, there is no evidence to indicate that the immune response plays a protective role against these tumors in the primary host [1, 23]. Indirect evidence for such a role has been sought, but has not been unequivocally obtained by attempting to show an inverse correlation between the antigenicity and the latent

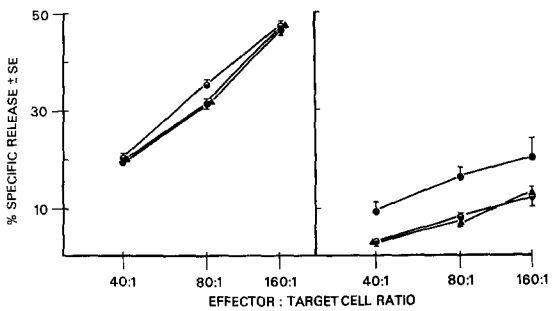

Fig. 1. NK activity of spleen cells from anti-IgM-treated mice (*left panel*) was compared with that of NRG-injected controls (*right panel*). The activity of the former is significantly higher, and unlike the latter, it does not diminish with the age of the animals. Spleens from 7- (●), 12- (◒), and 17- (▲) -week old mice were assayed. The target cells utilized were the YAC lymphoma, labelled with ^{51}Cr, at effector-to-target cell ratios as indicated on the *abscissa*. Incubation was for 4 h. The extent of cytotoxicity (*ordinate*) is expressed as percent specific ^{51}Cr release

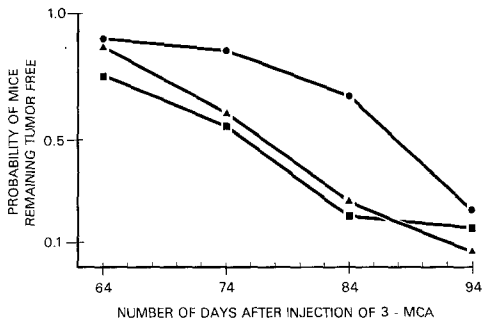

Fig. 2. Thirty normal mice (■), 22 animals given 5 mg of a rabbit anti-mouse IgM preparation by injection three times weekly from age 1 day (●), and 20 mice given equal amounts of normal rabbit globulin according to the same schedule (▲) received 0.5 mg methylcholanthrene at 7 weeks of age. The data presented represent the cumulative probability of mice remaining tumor-free at each time point illustrated, calculated from the percent tumor incidence by the life-table approach

period of chemically induced tumors [6, 14, 18, 24]. In a second approach, the incidence of tumors was studied in thymectomized and athymic nude mice. Although some reports claimed an increased incidence of sarcomas and lung adenomas in thymectomized mice [11, 19, 26], others failed to detect such a difference [2, 16, 17]. No increase in the incidence or decrease in latent period was seen in nu/nu mice compared with normal nu/+ controls; in fact, a lower tumor incidence was recently reported [9, 22, 25]. Thus experiments in both intact, and athymic mice were unable to reveal the existence of immunity against primary tumors. Natural resistance mechanisms have been shown to provide some protection against transplantation of small tumor inocula [12, 13], but again no clear evidence exists to implicate its involvement against primary malignancies. We approached this problem by examining the incidence of primary, MC-induced tumors in anti-IgM-treated mice, which we have shown to possess a heightened resistance to transplantable MC-induced tumors [3], as well as augmented NR mechanisms [4, 5] (Fig. 1). The results described in this communication have shown that the latent period of primary tumors in these mice is prolonged compared with that in untreated and NRG-treated animals (Fig. 2). This difference in latent period cannot be explained by altered nutritional or growth characteristics of anti-IgM-treated mice slowing down tumor growth, as the weight of these animals was comparable to that of those in the two control groups. Elimination of B lymphocytes also seems unlikely to remove the target cell for carcinogenesis by MC either. Equally unlikely is a direct effect on the tumor cells of the anti-IgM antibodies injected, as tests to detect such antibodies were uniformly negative [4]. Furthermore, such antibodies, if present in the anti-IgM preparation, would probably be natural antibodies also represented in the NRG preparation, which was found not to modify tumor incidence. Based on these considerations, we view the prolonged latent period as due to an active resistance, mediated by the immune system. Since anti-IgM-treated mice have no B lymphocytes, and their T cell immunity is not augmented [10], we suggest that the observed results are another manifestation of the heightened NR displayed by these mice. The failure of the heightened NR to modify the final tumor incidence may be explained by the high dose of carcinogen applied. This outcome is not unlike the one seen in NR to bone marrow, which can be overcome with increasing doses of parental bone marrow injected [8]. Alternatively, the increased latent period observed may reflect the preferential elimination of early tumors with high sensitivity to NR mechanisms, and selection of resistant tumors which may have arisen later. Further experiments are required to distinguish between the latter possibilities.

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References

1. Baldwin RW (1973) Immunological aspects of chemical carcinogenesis. Adv Cancer Res 18: 1
2. Balner H, Dersjant H (1966) Neonatal thymectomy and tumor induction with methylcholanthrene in mice. J Natl Cancer Inst 36: 513
3. Brodt P, Gordon J (1978) Anti-tumor immunity in B lymphocyte-deprived mice. I. Immunity to a chemically induced tumor. J Immunol 121: 359
4. Brodt P, Gordon J (1981) Anti-tumor immunity in B lymphocyte-deprived mice. II. In vitro studies. Cell Immunol 65: 20
5. Brodt P, Kongshavn P, Vargas F, Gordon J (1981) Increased natural host resistance mechanisms in B lymphocyte-deprived mice. J Reticuloendothel Soc 30: 283
6. Carbone G, Parmiani GJ (1975) Nonimmunogenic sarcomas induced by 3-methylcholanthrene treatment of murine fibroblasts in diffusion chambers. J Natl Cancer Inst 55: 1195
7. Colton T (1974) Longitudinal studies and the use of life table. In: Colton T (ed) Statistics in medicine. Little, Brown, Boston, pp 237–250
8. Cudkowicz G (1978) Natural resistance to foreign hemapoietic and leukemia grafts. In: Cudkowicz G, Landy M, Shearer GM (eds) Natural resistance systems against foreign cells, tumors and microbes. Academic Press, New York, pp 1–30
9. Gillette RW, Fox A (1975) The effect of T lymphocyte deficiency on tumor induction and growth. Cell Immunol 19: 328
10. Gordon J (1979) The B lymphocyte-deprived mouse as a tool in immunobiology. J Immunol Methods 25: 227
11. Grant GA, Miller JFAP (1965) Effect of neonatal thymectomy on the induction of sarcomata in C57BL mice. Nature 205: 1124
12. Haller O, Hansson M, Kiessling R, Wigzell H (1977) Role of nonconventional natural killer cells in resistance against syngeneic tumour cell in vivo. Nature 270: 609
13. Herberman RB, Djeu JY, Kay HD, Ortaldo JR, Riccardi C, Bonnard GD, Holden HT, Fagnani R, Santoni A, Puccetti P (1979) Natural killer cells: Characteristics and regulation of activity. Immunol Rev 44: 43
14. Kieler J, Radzikowski C, Moore J, Verich K (1972) Tumor genecity and isoimmunizing properties of C3H mouse cells undergoing (spontaneous) malignant conversion in vitro. J Nat Cancer Inst 48: 393
15. Klein G, Sjögren O, Klein E, Hellström KE (1960) Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res 30: 1561
16. Law LW (1966a) Studies of thymic function with emphasis on the role of the thymus in oncogenesis. Cancer Res 26: 551
17. Law LW (1966b) Immunologic responsiveness and the induction of experimental neoplasms. Cancer Res 26: 1121
18. Moore M, Williams DE (1973) Tumor-specific antigenicity of osteosarcomas induced in rodents by bone-seeking radionuclides. In: Saunders CL, Busch RH, Ballow JE, Hamlum DD (eds) Radionuclides carcinogenesis. U.S. Atomic Energy Commission Office of Information Services, Oakridge, Tennessee, pp 289–306
19. Nomoto K, Takeya K (1969) Immunologic properties of methylcholanthrene-induced sarcomas of neontally thymectomized mice. J Nat Cancer Inst 42: 445
20. Osmond DG, Gordon J (1979a) Characterization of small lymphocytes in the bone marrow of mice after prolonged treatment with anti-IgM antibodies. Cell Immunol 42: 188
21. Osmond DG, Gordon J (1979b) Differentiation of bone marrow lymphocytes after prolonged administration of anti-IgM antibodies to neonatal and adult mice. In: Baum SJ, Ledney GD (eds) Experimental hematology today 1979. Springer, New York, pp 129–138
22. Outzen HC, Custer RP, Eaton GJ, Prehn RT (1975) Spontaneous and induced tumor incidence in germfree "nude" mice. J Reticuloendothel Soc 17: 1
23. Prehn RT (1968) Tumor-specific antigens of putatively nonviral tumors. Cancer Res 28: 1326
24. Prehn RT (1970) Analysis of antigenic heterogeneity within individual 3-methylcholanthrene-induced mouse sarcomas. J Natl Cancer Inst 45: 1039
25. Stutman O (1974) Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice. Science 183: 534
26. Trainin N, Linker-Israeli M, Small M, Boiato-Chen L (1967) Enhancement of lung adenoma formation by neonatal thymectomy in mice treated with 7, 12, dimethylbenz(a)anthracene or urethan. Int J Cancer 2: 326

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