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Effect of Immune Response to Sheep Red Blood Cells on Plasmacytoma MOPC 104 E[1]

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ABSTRACT

BALB/c mice were given 1 × 10^6 MOPC 104E plasmacytoma cells i.v. to disseminate the neoplasm to various organs. Twenty-five days after implantation and at a time when the neoplastic B-cell clone was in the exponential growth phase, the mice were given i.p. injections of a mixture of antigens containing sheep red blood cells and levan. Each mouse was monitored simultaneously for immunoglobulin M (IgM) anti-dextran myeloma protein produced by the plasmacytoma and anti-sheep red blood cell hemolysin. The increase and decrease of these markers permit assessment of the expansion of the abnormal B-cell clone during the rise and fall of a normal B-cell clone in response to a specific antigen. The model was used to determine (*a*) the extent of the suppression of myeloma protein, (*b*) how long inhibition can be maintained, and (*c*) how soon it occurs after antigen is administered. The results showed that, as the IgM antibody response to sheep red blood cells begins to peak, it exerts a transient suppressive effect on either the MOPC 104E growth or on the cellular release of MOPC 104E IgM. The suppressive effect was noticeable 4 days after antigen administration for only 24 hr. These results indicated that plasmacytoma cells *in vivo* can recognize signals for either suppression of growth or release of the idiotypic MOPC 104E IgM and were not inconsistent with the view that myeloma may be the result of a defect in B-cell regulation.

INTRODUCTION

Myeloma represents an antibody-producing B-cell clone which appears to have escaped host regulation, and the clone is in a log expansion and in log phase of the antibody response. An antigen-induced immune response normally declines after log phase of antibody production. The mechanism of suppression is not yet well characterized. Suppression with soluble specific or nonspecific factors or cells of normal B-clones engaged in the immune response has been shown repeatedly in different systems (2, 4, 13). The depression of normal immunoglobulin levels in myeloma patients is ascribed to the formation of immunoregulatory cells (14) which synthesize suppressor substances (10). The kinetics of growth of the MOPC 104E plasmacytoma can be interrupted by drug treatment (8), and in some instances, regulation of the clone for greater than 200 days results *in vivo* (7).

In this study, we wished to determine if signals which control the normal host immune response could affect plasmacytoma growth. Experiments were designed to superimpose such signals on a growing plasmacytoma.

MATERIALS AND METHODS

Mice. Six-week-old female BALB/c mice were purchased from Laboratory Supply Company (Indianapolis, Ind) and maintained on standard laboratory mouse chow from Wayne Feed Company (Chicago, Ill.) and water *ad libitum.*

Murine Model. MOPC 104E is an induced plasmacytoma of BALB/c mice which produces a monoclonal IgM (15). M104E[3] has the unique property of reacting with bacterial Dextran B-1355 (11), while normal IgM does not (5).

Protocol for MOPC 104E Plasmacytoma Growth and Immune Response to SRBC. A group of mice were given i.v. injections of 1 × 10^6 MOPC 104E cells. The mice were followed for an increase in circulating M104E with time. The MOPC 104E-bearing mice were divided into 4 groups of 5 animals each. Twenty-five days after implantation, one group of mice was treated with SRBC (0.1 ml of 50% SRBC), the second group was given levan (100 μg/mouse), and the third group was treated with both SRBC and levan. Levan served as adjuvant, producing a more intense IgM antibody response to SRBC. Polyclonal antigens given along with SRBC were shown to augment the 19S IgM plaque response (12). Five MOPC 104E-bearing mice served as untreated tumor controls. Three groups of normal BALB/c mice were also immunized with SRBC, levan, or both and served as normal immune response controls. Eighty μl of blood were taken every day from Day 2 to 6 after immunization, and the plasma was followed for both M104E and hemolysin, as described below. The antibody response to levan, while not tested in this study, did not interfere with the detection of M104E antibodies to dextran, which were produced by the plasmacytoma. The MOPC 104E protein reacts with the α1,3 linkages of the dextran and not the β2,6 or β2,1 linkages of levan (18).

Quantitation of M104E and Anti-SRBC IgM. Mice were weighed, and approximately 80 μl of blood were taken in a microhematocrit tube from the tail vein. The total plasma volume per mouse was determined from these 2 values. Each time a sample was taken, the total plasma volume per mouse was established. Based on plasma volume and M104E levels in the plasma, the total M104E in the circulation of the animal and the number of MOPC 104E cells could be calculated for each animal (6).

SRBC conjugated with dextran were specifically lysed in the presence of M104E and complement (5). The procedures for measuring M104E in the plasma or serum by radial hemolysis in gel were described previously (5, 9). Both M104E and hemolysin activity were quantitated as described briefly below. Hemolysin activity was directly quantitated on SRBC plates; M104E, antidextran antibodies did not react with SRBC or

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[3] The abbreviations used are: M104E, MOPC 104E IgM; SRBC, sheep red blood cells; hemolysin, anti-sheep red blood cell IgM antibody.

interfere with SRBC lysis. M104E was quantitated in plates containing dextran-conjugated SRBC in the presence of excess SRBC stroma which blocked hemolysin but did not inhibit anti-dextran IgM lysis. To minimize the variability of daily titrations, all plasma samples were stored, and assays were carried out 1 time after the conclusion of the experiment.

RESULTS

Although the number of MOPC 104E cells administered i.v. and the amount of antigen given were the same for animals in each group, individual differences of plasmacytoma body burden and immune response occurred. Therefore, in the measurement of response of non-tumor-bearing BALB/c mice to SRBC, levan, and their combination, all animals were monitored daily from Days 2 to 6 after immunization individually, and the average per group ± S.E. is given (Table 1). Mice treated only with levan gave negligible response to Dextran B-1355 and had a similarly weak response to SRBC with a mean antibody level of 2.4 ± 0.7 μg/mouse on the fifth day. Animals treated with only SRBC gave a normal response for SRBC with a mean antibody value of 265.3 ± 85.3 μg/mouse on Day 5. In contrast, animals given SRBC and levan demonstrated an augmented response to SRBC with a mean antibody level of 460.3 ± 27.7 μg/mouse.

To study further the influence of MOPC 104E burden in the host on the immune response, BALB/c mice were given injections of 1×10^6 MOPC 104E cells 25 days prior to immunization with SRBC-levan. The plasmacytoma was allowed to progress until it was in exponential growth. On Day 25, the animals were given the antigen mixture, which was shown to augment hemolysin response. Both the hemolysin level and the course of the plasmacytoma growth were monitored by quantitating the hemolytic antibodies and M104E marker in the circulation. The M104E values on the day prior to immunization ranged from 44.8 to 280.0 μg/mouse. In these animals, the mean circulating M104E values rose over the period tested, and by the seventh day, they were approximately 5000 μg/mouse. The hemolysin levels in these animals increased and peaked on Day 5 (mean value, 426 μg/mouse). The quantity of hemolysin produced on the fifth day was similar to that of normal BALB/c mice given the SRBC-levan antigen combination and was higher than that of normal BALB/c mice given SRBC without levan. M104E is produced in large quantities by MOPC 104E cells and was not due to anti-dextran antibodies induced by the polyclonal antigen, levan.

From each M104E value, the number of plasmacytoma cells per animal was determined. By using the weight of each animal, the plasmacytoma burden of the mice over the time period was determined. The quantitative relationship of the rate of growth of the M104E, as measured by the M104E and the course of the immune response over the same time period, was determined (Chart 1). The results show that, between Days 4 and 5 when the hemolysin response began to peak, an inhibitory action on either the rate of release of M104E by the plasmacytoma cells or its rate of growth (doubling time) was affected.

In the SRBC-levan-treated normal BALB/c mice, the mean SRBC responses were 460.3 ± 27.7 and 506.90 ± 156.70 μg/mouse on Days 5 and 6, respectively. In contrast, mice treated similarly with an average MOPC 104E burden of 4.06% (Day 5) and 5.48% (Day 6) had mean hemolysin levels of 426.2 ± 72.3 and 188.4 ± 7.5 μg/mouse, respectively. Mice with 5.48% burden had 63% less antibodies than did normal BALB/c mice given the same antigen. Although the hemolysin level was not affected significantly on Day 5 of the immune response, there was a definite decline of hemolysin on Day 6 in MOPC 104E-bearing mice.

DISCUSSION

It has been shown that plasma cell tumors suppress the immune response to a variety of antigens (1, 19, 20). The studies presented here show that a delay in the growth rate of the MOPC 104E plasma cell tumor takes place during a strong primary response to antigens. The course of the growth and the host immune capacity are interrelated in that one affects the other and *vice versa*. We show that several simultaneous time-related events are taking place in the host when the antigen is given to an animal with a plasmacytoma. The first observation is the SRBC-levan mixture (used as a nonspecific antigen) caused a transient inhibition of the idiotypic IgM produced by the plasmacytoma. Whether this indicates a suppression of the shedding of surface IgM into the circulation is not entirely clear at this time. If we assume that the M104E synthesis is interrupted as a consequence of the normal primary IgM response being initiated by spleen cells to the antigen, then it is clear that, as the SRBC IgM response begins to peak, strong selective pressures are brought to bear on the plasmacytoma cell population and that a transient suppression of tumor product results. MOPC 104E cells can detect either humoral factors and/or a signal from cells engaged in the response to SRBC-levan. During the period when the response to SRBC is at its peak, either the shedding of the M104E or inhibition of the proliferation of the clone takes place for a brief period. This selective control is short-lived in that, as the immune response to SRBC declines, production of M104E is resumed.

A second observation is that, as the size of the MOPC 104E (percentage of burden) increases, the total amount of antibody

Table 1

Hemolysin response of normal and MOPC 104E tumor-immunized mice with SRBC-levan

Mice were followed for μg IgM antibody response by radial hemolysis in gel method on Days 2 to 6 postimmunization.

	μg IgM antibody/mouse				
Mice	Day 2	Day 3	Day 4	Day 5	Day 6
Normal	1.5 ± 0.2[a]	12.9 ± 0.6	134.1 ± 22.2	460.3 ± 27.7	506.9 ± 157.7
MOPC 104E[b]	0.1 ± 0.0	8.2 ± 2.5	144.2 ± 11.0	426.2 ± 72.3	188.4 ± 7.5

[a] Mean ± S.E.
[b] The day of immunization corresponds to Day 25 post-tumor implantation.

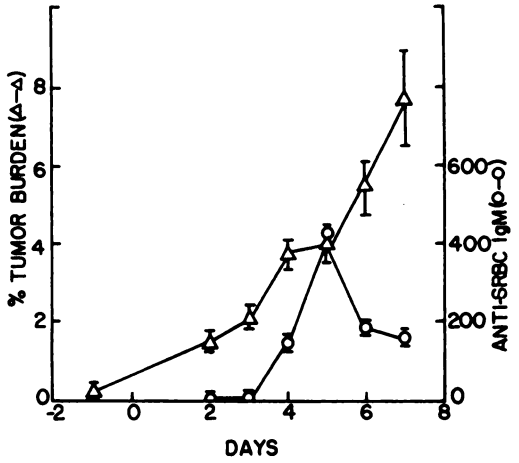

Chart 1. Effect of the immune response to SRBC on the MOPC 104E plasmacytoma. A linear plot of the MOPC 104E burden per mouse (△) and μg of anti-SRBC IgM per mouse (○) *versus* days. Day 0 indicated the day SRBC-levan was given i.p. *Bars*, S.E.

produced in the circulation to SRBC decreases. Moreover, the response is not sustained as compared to the normal animals. This is substantiated by the amount of hemolysin quantitated in animals with MOPC 104E at 6 days (188.4 ± 7.5 μg of SRBC antibody per mouse) compared with that of normal BALB/c mice (506.9 ± 156.7 μg of SRBC antibody per mouse). The animals with MOPC 104E produce 63% less IgM antibody to SRBC at this time than do normal BALB/c mice given the same antigen. Similar observations showing an immunodeficiency in myeloma have been made in mice (19, 20) and humans (1).

In the normal immune response, both humoral (17) and cellular suppressor mechanisms (3) operate simultaneously to maintain a regulatory balance. In multiple myeloma, suppressive effects are imposed on normal cells, and the tumor cell has circumvented this immunoregulatory control. Regulation of growth of the MOPC 104E clone has been accomplished *in vivo* by drug therapy (7). Drug therapy reduces the clone size, and the conditions are not unlike those used to reduce tolerance with cyclophosphamide in a host sensitized previously to antigens. The long-term regulation of the B-cell clone after drug treatment appears to differ from the transient suppression demonstrated by our study. It indicates, however, that the autonomous MOPC 104E cells possess receptors that allow them to respond to signals of suppression. This response has been detected *in vivo* by a functional decrease in IgM production by the cells. The nature of the cell surface receptor responsible for receiving signals and the humoral mediator(s) or cells responsible for transmitting the signals still have to be identified. It is apparent that, if the factor is humoral in nature, it must have a short half-life and that its effect lasts in the circulation between Days 4 and 5 after antigen administration. Multiple myeloma is a disease that affects the aged, and with aging, there is increasing aberration of immunoregulatory controls; longitudinal studies comparing development of immune defects with the clinical course in humans are of particular interest. In the NZB mice, the correlation among aging, immunoregulatory loss, and the incidence of plasmacytomas has been demonstrated (16).

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