Age-related Changes of Natural Antitumor Resistance in Spontaneously Hypertensive Rats with T-Cell Depression

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ABSTRACT

We investigated the relationship between age-related changes in natural resistance and antitumor effects using a spontaneously hypertensive rat (SHR rat) strain which shows a progressive decline of the number of T-cells and their functions as a result of aging.

The growth of a weakly antigenic mammary adenocarcinoma SST-2 was significantly suppressed in SHR rats ages 2 and 3 months, whereas in SHR rats ages 1 or 8 months no suppression of the tumor growth was observed. Splenic natural killer cell activity among the SHR rats was still low at 1 month, when the T-cell function is relatively intact; it reached a maximum level at 3 months and thereafter rapidly decreased. On the other hand, the cytostatic activity of peritoneal macrophages, which is also low at 1 month and becomes high at 3 months, thereafter remained at high levels until 8 months of age. That is, the kinetics of natural killer cell activity during the aging processes runs parallel to the function of suppressing tumor growth. Treatment with anti-asialomonoganglioside antiserum abrogated the suppressive activity of SST-2 tumor growth in 3-month-old SHR rats. Treatment with double stranded RNA polyinosinate-polycytidylate, an interferon inducer, produced significant suppression of the tumor growth in SHR rats ages 3 and 8 months. These results suggest that the participation of natural killer cells is a principal effector mechanism in the suppression of SST-2 tumor growth in SHR rats ages 2 and 3 months.

INTRODUCTION

It is well known that immunoreactivity varies with increasing age (1-4). Most of the available data suggest that the principal deficit in aged mice is based on an alteration in the thymus-dependent immune system (5-8), although some age-related deficiencies in the B-cell population have also been reported (9). The immune capacity, the in vitro response to the T-cell mitogens, and the production of antibody-forming cells in response to sheep red blood cells have been shown to decline with age (10-12). It has also been reported that the thymus-independent natural resistance mediated by natural killer cells, macrophages, and polymorphonuclear leukocytes differs between young and aged animals (13-15).

We recently reported that a strain of SHR rats, which has been established as an animal model for human essential hypertension by Okamoto and Aoki (16), undergoes a progressive decline of T-cell functions owing to aging; i.e., the T-cell function remains intact at 1 month old, but after 2 months the function is rapidly depressed (17). This deficit in SHR rats is closely associated with an early appearance of a natural thymocytotoxic autoantibody (18) and a deficit in SHR rats is closely associated with an early appearance of a natural thymocytotoxic autoantibody (18) and a deficit in SHR rats ages 2 and 3 months.

MATERIALS AND METHODS

Animals. A closed colony of SHR rats was obtained from the Nippon Rat Co., Inc., Urawa, Japan, and 1- to 12-month-old male and female SHR rats were used throughout the experiments.

Tumor. A transplantable mammary adenocarcinoma (SST-2) was derived from a 6-month-old female SHR rat. This tumor is weakly antigenic and produces a high incidence of lung metastasis in SHR rats. SHR rats, 1 to 8 months old, were inoculated s.c. with 2 x 10⁶ SST-2 tumor cells/rat (minimum take dose) or 1 x 10⁶ cells/rat (sufficient take dose). After tumor cell inoculation, we measured the tumor diameter twice a week and calculated the growth rate of the tumor.

Administration of Anti-ASGM₁ Antiserum. To abolish NK cell activity, SHR rats were given i.v. injections of rabbit anti-ASGM₁ antiserum (diluted 1:20) 6 times at 5-day intervals from 1 day before the tumor cell inoculation. Control rats were treated with 1:15 dilution of normal rabbit serum.

Assay of NK Cell Activity. The killing activity of splenic NK cells was measured by means of a 4-h ⁵¹Cr release assay. Target cells, YAC-1, were prelabeled for 1 h at 37°C with ⁵¹Cr, washed three times in cold minimum essential medium, and allowed to leak for at least 1 h at 37°C in RPMI 1640 plus 10% fetal bovine serum. Appropriate numbers of spleen cells were mixed with 1 x 10⁵ ⁵¹Cr-labeled target cells and were then seeded in 96-well round bottomed microtitre plates. The plates were incubated for 4 h at 37°C and the radioactivity of the supernatant was counted in a Beckman gamma counter. The percentage of cytotoxicity was calculated as

\[
\% \text{ of specific release} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}} \times 100
\]

Assay of Macrophage-mediated Cytostasis. The in vitro cytostatic activity of peritoneal macrophages was determined by using the [H]-dThd incorporation inhibition test. BMT-11 tumor cells (1 x 10⁹) in 0.2 ml of RPMI 1640 with 10% fetal bovine serum were cultivated on minimum essential medium, and allowed to leak for at least 1 h at 37°C in RPMI 1640 plus 10% fetal bovine serum. Appropriate numbers of spleen cells were mixed with 1 x 10⁶ [H]-dThd-labeled target cells and were then seeded in 96-well round bottomed microtitre plates. The plates were incubated for 4 h at 37°C and the radioactivity of the supernatant was counted in a Beckman gamma counter. Results were expressed as the mean cpm and as the percentage of inhibition of tumor cell proliferation calculated as

\[
\% \text{ of inhibition} = \frac{\text{cpm}_1 - \text{cpm}_2}{\text{cpm}_1} \times 100
\]

where \(\text{cpm}_1\) is the mean cpm in cultures with target cell alone and \(\text{cpm}_2\) is the mean cpm in cultures containing macrophages with target cells.

Administration of Poly(I)-Poly(C). Rats were given i.p. injections of 5 mg/kg poly(I)·poly(C), double strand RNA (P-L Biochemicals, Inc., Lab of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo 060, Japan)
Milwaukee, WI), in 0.5 ml phosphate-buffered saline 4 times at weekly intervals from 1 day before tumor cell inoculation.

RESULTS

Changes of Growth Curves of SST-2 Tumor in SHR Rats as a Result of Aging. Fig. 1 shows the growth curves of SST-2 tumor in 4 different age groups of SHR rats (10 rats/group) after s.c. inoculation of a minimum take dose of $2 \times 10^5$ tumor cells. The growth of the SST-2 tumor was strongly suppressed in 3-month-old SHR rats and was also significantly suppressed in 2-month-old rats, compared with two other groups of SHR rats ages 1 and 8 months. On the other hand, when a sufficient take dose (1 x $10^6$ cells/rat) of SST-2 tumor cells was inoculated, the growth of the tumor was not suppressed in any of the groups (data not presented).

Changes of Splenic NK Cell Activity in SHR Rats as a Result of Aging. Fig. 2 shows the age-related changes of NK cell activity against YAC-1 cells in spleens of SHR rats (4-5 rats/group) ages 1-12 months as detected by $^{51}$Cr release assay. The splenic NK cell activity of SHR rats was still low at 1 month of age; it reached a maximum level at 3 months of age; after 3 months of age, however, the activity rapidly decreased.

Changes of Cytostatic Activity of Macrophages in SHR Rats by Aging. We investigated the age-related changes of cytostatic activity of macrophages in SHR rats (4-5 rats/group) ages 1-12 months by means of a $[^{3}H]$dThd incorporation inhibition test. Fig. 3 shows that the cytostatic activity of peritoneal macrophages against BMT-11 tumor cells was low in 1-month-old SHR rats but rose to a high level in 3-month-old SHR rats, thereafter it remained at high levels in SHR rats until they were 8 months old.

Effect of Treatment with Anti-ASGM$\gamma$ Antiserum. Fig. 1 shows that the growth of SST-2 tumor was strongly suppressed in 3-month-old SHR rats when compared with SHR rats ages 1 and 8 months. In order to identify the effector cells active in the suppression of the tumor growth, groups of SHR rats (10 rats/group) ages 1, 3, and 8 months were given i.v. injections of anti-ASGM$\gamma$ antiserum 6 times a day at 5-day intervals from 1 day before the tumor cell inoculation. The results are presented in Fig. 4. Tumor growth was markedly enhanced in the treated SHR 3-month-old rats and was also significantly enhanced in the treated 8-month-old SHR rats in its initial stages when compared with those of age-matched controls. However, no different tumor growth was observed between the 1-month-old treated and control rats.

Effect of Treatment with Poly(I)-Poly(C). Fig. 5 shows the NK activity of spleen cells obtained from SHR rats treated with poly(I)-poly(C) 1 day before the assay. The marked increase in NK activity, although modest in the treated 1-month-old SHR rats, was observed in 3- and 8-month-old treated rats when compared to nontreated age-matched controls. The effects of poly(I)-poly(C) treatment on the growth of SST-2 in SHR rats reached a maximum level at 3 months of age; after 3 months of age, however, the activity rapidly decreased.

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cells in specific pathogen-free W/Fu rats is not age restricted, ever, Rees et al. (21) reported that, despite some variability, clear evidence indicates the participation of NK cells as a main effector mechanism in the suppression of SST-2 tumor growth. How this is controlled by activated NK cells, even though, owing to aging, it is difficult for the T-cell mediated immune systems to recognize weakly antigenic or nonantigenic tumors in the state of T-cell suppression. Finally, the results of the present experiment suggest that the growth of a low immunogenic tumor such as SST-2 can be controlled by activated NK cells, even though, owing to aging, it is difficult for the T-cell mediated immune systems to recognize weakly antigenic or nonantigenic tumors in the state of T-cell suppression.

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