Participation of the Immune System in Regression of a Rat Mc7 Sarcoma by Hyperthermia

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ABSTRACT

Participation of the host immune response in eradication of tumor by hyperthermia has been suspected for a long time. The effect of local tumor heating on the immunocompetence of rats bearing Mc7 sarcoma was studied. Following heat treatment of 1- to 1.5-ml foot tumors at 43° for 2 hr, regression of primary tumors resulted in host cure (15 of 21; 71%), and this was accompanied by an increased skin response to both 3 m KCl extract of Mc7 and dinitrochlorobenzene as well as in elevation of antibody to bovine serum albumin. Increased levels of antitumor antibody were not detected. Animals that were cured by hyperthermia showed no sign of metastatic tumor in lymph nodes and lungs; most control animals at the time of heat treatment had secondary tumor deposits in lymph nodes and lungs. Tumor regression after curative heating did not occur in rats (0 of 10) treated by whole-body X-rays (150 R; 3 times) plus cortisone acetate (60 mg/kg; 4 times; s.c.), and the tumor cure rate was reduced (9 of 21; 43%) by blocking macrophage activity with silica (1 g/kg; i.v. and i.p.). Also, these rats (43%) succumbed to tumor challenge with 2.5 × 10⁶ Mc7 cells; 50% of the heat-cured animals not given silica consistently rejected this challenge dose. The results imply that immunostimulation comprising mainly T-cells and macrophages plays a major part in tumor regression by hyperthermia.

INTRODUCTION

Hyperthermia (temperatures ≥42°) is widely used in the treatment of cancer (see Refs. 6 and 13). The mechanism whereby heat exerts its selective destructive effect on cancer cells is not fully understood. Effects have been observed on lysosomes, cell pH, protein, nucleic acid synthesis, and tumor blood flow (see Refs. 5 and 6). It has been suspected for a long time that the host immune system may be actively involved in tumor regression that occurs after thermotherapy. Recently, we reported that, following local heating in VX2-bearing rabbits, regression of primary leg tumor and disappearance of metastases in lymph nodes and lungs were accompanied by an increase in both cellular and humoral immune competence in vivo. The cured animals became immune to further tumor challenge with 30 × 10⁶ VX2 cells; 1 × 10⁶ VX2 cells killed all untreated animals (7, 9). The present study in rats is an extension of our findings in rabbits with a view to determine if tumor cure by hyperthermia could occur in the absence of a functioning immune system.

MATERIALS AND METHODS

Sarcoma Mc7 was induced in female inbred Wistar rats by s.c. injection of 3-methylcholanthrene in trioctanoin (1). For the present work, Mc7 tumor (100 mg) was transplanted into the dorsum of left hind foot of 175- to 200-g rats. Tumor volumes were calculated from caliper measurements made in the anteroposterior, lateral, and vertical planes of the foot, allowance being made for the normal foot thickness before inoculation of tumor cells.

Immune Response Assay. Cell-mediated immune response was measured by the delayed hypersensitivity skin reaction to 3 m KCl extract of Mc7 tumor and to DNBC. The method used for preparing Mc7 extract was the same as for the rabbit VX2 tumor (9). The skin response to tumor protein was determined by change in footpad thickness at 24 hr after injecting 75 μg of tumor protein in 0.1 ml of 0.9% NaCl solution. Details of the procedure for sensitizing, challenging, and measuring the skin response to DNBC in rats were similar to those for rabbits (8). For sensitization, DNBC (20 mg/kg; 2 mg/50 μl/100 g body weight) in acetone was spread within an area of approximately 2.5 sq cm of the shaved skin of the back, about 3 cm below the neck of the rat. The first DNBC challenge (1 mg/kg; 0.1 mg/15 μl/100 g body weight) on rat ear was performed 11 days later. The change in ear thickness at 24 hr after DNBC challenge was measured with a micrometer.

Antitumor and anti-BSA antibody titers were determined by passive hemagglutination test as described previously (8, 9). For primary response, BSA (5 mg/kg; 0.5 mg/0.5 ml/100 g body weight) was injected with complete Freund’s adjuvant in the right hind leg muscle. The animals were bled weekly from the jugular vein. The second BSA injection was given about 30 min before the tumor was treated by water bath heating.

Immunosuppression by Whole-Body Irradiation. Rats were exposed to whole-body 150 R X-rays irradiation (30 R/min) within 2 hr after hyperthermia. Total-body irradiation was repeated at 48 and 96 hr later. The rats were then treated with cortisone acetate (Boots Pure Drugs, Nottingham, England), 60 mg/kg, given s.c. at 24, 48, 96, and 120 hr after the last X-ray treatment.

Silica Treatment. Animals were treated 3 times with a suspension of silica at a dose of 1 g/kg body weight (particle size < 5 μ; Dorentrup Quartz No. 12, from Dr. M. V. Pimm, Cancer Research Campaign Laboratories, University of Nottingham, England). The preparation was heat sterilized, suspended in 0.9% NaCl solution (100 mg/ml), and exposed to 20 sec of ultrasound (20 kHz) from an MSE Ultrasonicator immediately before use to ensure absolute dispersion. For each of the 3 injections, 90% of the required dose of silica was given i.p. and the rest i.v., starting 1 day before hyperthermia with 2 further doses given at 5- to 7-day intervals. The macrophage activity in rats was determined by the rate of clearance of colloidal carbon from blood as described before (10).
Hyperthermic Treatment of the Mc7 Tumor. For heat treatment of 1- to 1.5-ml foot tumors, the rats were anesthetized with 0.1 ml of a 1:5 dilution of Nembutal veterinary given i.p. (60 mg pentobarbitone sodium per ml; May and Baker Ltd., Degenham, England) per 50 g body weight. The tumors were treated by water bath immersion at 43° for 2 hr. Intratumor temperatures during the treatment were monitored continuously with thermocouple needle probes connected to a Doric digital meter (Trendicator 400; Doric Scientific Corp., San Diego, Calif.). Details for water bath and temperature measurements were as before (2, 4).

Experimental Design. The schedule used in monitoring the response of the immune system and for producing immunosuppression in tumor-bearing rats treated by curative hyperthermia is shown in Chart 1. The timing of BSA and DNCB sensitization, of challenge with tumor extract, of immunosuppression with silica and X-rays plus cortisone acetate, and of tumor heating was in relation to tumor cell inoculation (Day 0).

RESULTS

Tumor Heating and Host Cure

Chart 2 details the effect of local heating on primary tumor volume in 12 of 21 treated rats. Chart 2B also shows mean tumor growth in 15 of 27 untreated control animals. From an initial transplant of a 0.1-ml tumor into the left hind foot of the rat, the tumor reached a volume of 1 to 1.5 ml by 13 days. Ninety % of the untreated rats died by 30 days with métastases in regional and distant lymph nodes and lungs. Three of 27 untreated animals showed spontaneous regression within 10
days after tumor transplant. Tumors (1 to 1.5 ml) were treated by water bath heating at 43° for 2 hr, which resulted in a cure rate of 71% (15 of 21 rats cured). Detailed histological analysis was performed on lymph nodes and lungs in 14 control rats bearing 1- to 1.5-ml foot tumors Days 11 to 13 after the tumor inoculation. The animals were selected at random during the entire study period. Gross enlargement of ipsilateral and contralateral popliteal and inguinal lymph nodes, confirmed microscopically by the presence of tumor foci, was observed in all 14 animals. Twelve of the 14 rats also showed pulmonary metastases and the presence of tumor in auxiliary lymph nodes. Tactual examination of the popliteal lymph nodes on Days 11 to 13 in the above 14 control animals and also in 21 rats that were treated by hyperthermia revealed gross enlargement due to the presence of tumor. Lymph nodes and lungs from the cured animals, when examined microscopically on Day 60 (47 to 49 days after tumor heating), failed to show any sign of tumor growth, suggesting regression of metastases after curative local heating. Fifty % of the cured rats rejected a challenge of 2.5 x 10³ Mc7 cells given into the right hind foot; all normal rats died with this dose of tumor cells (see Table 1).

Effect of Hyperthermia on the Immune System

One hundred % of the rats, normal or tumor bearing, responded to DNCB regimen used. The change in ear thickness (cm x 10³) after first DNCB challenge in 48 tumor-bearing rats was 11.04 ± 2.87 (S.D.) and that in 12 normal non-tumor-bearing animals was 11.00 ± 1.63. The change in footpad thickness (cm x 10³) after first challenge with tumor extract in the above 48 tumor-bearing animals was 16.50 ± 2.3. The initial response of tumor-bearing rats to DNCB or to tumor extract was therefore uniform among rats. However, the quantitative difference in response to subsequent DNCB or tumor extract challenges among untreated animals or heat-treated rats was up to 5-fold. These findings in rats were analogous to those of our previous work with rabbit VX2 carcinoma (see Refs. 8 and 9). The response to DNCB and to tumor extract in rats has therefore been plotted as the percentage of change to the initial response (i.e., tumor regression led to decreased immune response and vice versa).

Cellular Response: Skin Tests. Chart 3 shows that in untreated rats there was a progressive decrease in skin response to tumor extract as the tumor increased in volume and the animals died. Following curative hyperthermia, tumor regression was accompanied by a significant increase in skin response to tumor extract. On the other hand, the response to a 3 m KCI extract of normal rat liver (75 µg protein), when tested in animals with regressing tumor, was always negative. Chart 4 illustrates the effect of curative tumor heating on DNCB response in rats. In untreated rats in which tumors grew progressively, the response decreased with time. Animals in which the tumor grew more slowly showed a well-maintained DNCB response. Successful tumor heating resulted in recovery of DNCB response. This augmented T-cell function was less marked when compared with skin response to Mc7 tumor extract (Chart 3).

Humoral Response. Antitumor antibody was not detected in rat sera using passive hemagglutination, Ouchterlony immunodiffusion, or immunoelectrophoresis techniques. However, after curative tumor heating, the antibody levels to a foreign antigen BSA were maintained or increased from about 1/10 to up to 1/60 (Chart 5). The pattern of response in rats that failed to respond to heat treatment was similar to that of untreated rats, i.e., after an initial increase in antibody titer followed by progressive decrease as tumors increased in volume resulting in animal death.

Effect of Host Immunosuppression on Curative Tumor Heating

After tumor heating, when rats were subjected to total-body irradiation plus cortisone acetate, circulating WBC and lymphocyte counts decreased to less than 10% of normal values and remained at less than 50% of normal for 3 weeks or more after therapy. Growth rate of primary tumor in such immunosuppressed rats was merely retarded, and the host cure rate was
reduced from 71% to zero (Table 1). There was a concomitant decrease in host immunocompetence, as monitored by skin testing with Mc7 extract and DNCB or anti-BSA antibody titers. Furthermore, when rats that were cured by heat treatment alone were immunsuppressed as above, all the animals produced lethal tumors on rechallenge with 2.5 \times 10^6 Mc7 cells. Tumor growth in this group of rats was accompanied by decreased T- and B-cell responses compared to nonsuppressed animals that rejected tumor challenge.

To assess the requirement for host phagocytic cells in tumor regression by locally applied heat, tests were carried out in rats treated with particulate silica, a specific macrophage poison. Initial results showed that repeated injections of silica in rats maintained the macrophage activity, as determined by the clearance of colloidal carbon from blood, to about 50% of normal values. Under these conditions when Mc7-bearing rats were treated by heat, the cure rate of the animals decreased from 71 to 43% (p < 0.062, \chi^2 test). However, all heat-plus-silica-treated rats (43%) died from challenge with 2.5 \times 10^6 Mc7 cells; 50% of the cured animals not given silica rejected this challenge dose (Table 1).

DISCUSSION

The results demonstrated that curative heating of the syngeneic Mc7 sarcoma at 43° for 2 hr was accompanied by an increase in both specific (skin response to tumor extract) and nonspecific (skin response to DNCB and anti-BSA antibody) host immune response. When the immune competence of the rat was depressed by whole-body irradiation plus cortisone acetate or by silica (a macrophage toxin), tumors failed to regress after heat therapy, and the host cure rate decreased. An intact host immune response would therefore appear to be necessary in order to obtain maximum benefit from tumor heating.

In support of the present findings, some recent studies have indicated that host cure by local hyperthermia involves participation of the immune system. Shah and Dickson (7, 9) found that, following local heating of allogeneic rabbit VX2 carcinoma at 47–50° for 30 min, regression of the primary leg tumor and metastases in lymph nodes and lungs resulted in host cure with increased immunocompetence of the animal. Curative tumor heating was accompanied by an increase in delayed hypersensitivity skin reaction to VX2 tumor extract and to DNCB, and there was a 100-fold increase in the antitumor and anti-BSA antibody in the rabbit. In animals that failed to respond to heat treatment (25%) or in untreated animals, progressive increase in tumor growth was associated with decreased skin reactions and antibody titers of 1/10 or less (7, 9). Szmigielski and Janiak (14) reported that local microwave heating of Guerin carcinoma at 43° for 45 min on 3 occasions at 48-hr intervals.
caused a significantly increased reactivity of host spleen cells to phytohemagglutinin and to mitomycin-inhibited tumor cells, as well as increased cytotoxicity of spleen lymphocytes against $^{51}$Cr-labeled Guerin tumor cells. Elevation of anti-BSA antibody also occurred in the heat-treated animals. Shahlin et al. (12) observed that, in patients with melanoma, hyperthermic perfusion at 40° with L-phenylalanine mustard (melphalan) increased the cytotoxic effect of the patients’ lymphocytes and plasma against the autologous tumor cells in vitro from 10% inhibition before treatment to 80 to 85% inhibition at 2 weeks after treatment. The last 2 studies (12, 14) give no details on control target cells used, lymphocyte/target cell ratio used, or what constituted tumor cell inhibition for in vitro cytotoxicity assay, and hence, the tumor directed specificity of the increased lymphocyte or antibody activity is difficult to assess.

Several reports in the literature suggest that the host response to hyperthermia may be nonspecific in nature, mainly involving macrophages of the reticuloendothelial system. Impairment of rat macrophages with silica (present work) or colloidal carbon (10) reduced the heat response of MC7 tumor. All the cured animals (Table 1, 43%) produced tumors on subsequent MC7 challenge compared to a 50% take in cured rats not treated with silica. Shah and Dickson (11) observed that, when macrophages were stimulated with Corynebacterium parvum given i.v., the MC7 tumor cure rate increased from 50% to 85% at 24 hr after hyperthermia with 2 further doses given at 5- to 7-day intervals.

Rats with whole-body X-rays (150 R 3 times at 48-hr intervals) within 2 hr after hyperthermia. Cortisone acetate (60 mg/kg body weight 4 times at 48-hr intervals) was given s.c., starting at 24 hr after third X-ray treatment.

It is therefore apparent that, in both allogeneic and syngeneic animal tumor systems, regression of the primary tumor and distant metastases after curative local hyperthermia is accompanied by an increased state of host immunocompetence mainly involving cellular immunity. The findings suggest that immunostimulation comprising T-cells and macrophages play a major part in eradication of tumors by hyperthermia. In the present study, antitumor antibody was not detected after curative heating. The role of antibody in host immunocompetence against cancer is doubtful, especially in view of recent work by Dennick et al. (3) showing that the presence of tumor-specific antibody against a rat D23 hepatocellular carcinoma was not related to the ability of the animal to reject tumors.

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