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Immunology

Reduction of syngeneic tumor growth by an anti-I-J alloantiserum

(tumor growth/*H-2* complex/*I* region)

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ABSTRACT **Highly significant suppression of the growth of S1509a and Sa-I syngeneic sarcomas was observed in A/J mice following daily intravenous injections of 2 μl of anti-I-J^k alloantiserum. This effect persisted as long as the anti-I-J^k serum was administered (day 15). In contrast, a control anti-I-J^s serum had no discernible effect on the growth of the S1509a tumor. The inhibitory activity of the anti-I-J^k serum on the growth of the tumor was absorbed specifically by B10·BR spleen cells bearing *I-*J^k determinants. In other experiments, we established that A/J mice treated with anti-I-J^k serum, according to the protocol described above, are no longer a source of tumor-specific suppressor cells for adoptive transfer into immune tumor-bearing recipient mice. We conclude that anti-I-J^k serum inhibits tumor growth in A/J mice by abolishing tumor-specific suppressor activity.**

The failure of the host to mount an effective immune response against growing syngeneic, yet demonstrably antigenic, tumors has been attributed to complex tumor and host factors that protect the tumor against the immune defenses of the host. One of the mechanisms favoring tumor growth is the development of specific suppressor thymus-derived (T) cells. Recent studies of the host reaction to murine syngeneic tumors have established that a progressively growing methylchloranthrene-induced fibrosarcoma stimulates the development, within the lymphoid organs, of T cells capable of suppressing the immune rejection of the tumor (1, 2). Furthermore, such suppressor T cells were shown to produce tumor-specific *H-2* coded products with tumor-enhancing properties (3). This tumor-specific suppressor T cell factor and other specific T cell factors capable of suppressing antibody responses (4–6) or contact sensitivity (7, 8) have similar biological and immunochemical properties. Suppressor T cells (9) and specific suppressor factors (8, 10, 11) bear determinants coded for by the *I-J* subregion of the *H-2* complex not displayed on other T cells or other immunological regulatory factors.

Anti-I-J antisera could, therefore, be expected to behave as highly effective antisuppressor agents *in vivo*. Recent studies by Pierres *et al.* (12) in our laboratory have investigated the effect of anti-I-J alloantisera, administered intravenously, on the antibody response to suboptimal doses of sheep erythrocytes (SRBC). The injection of microliter quantities of anti-I-J^k antisera regularly and significantly enhanced IgM and IgG responses of A/J mice to SRBC (12). Prompted by these results, we investigated the effect of anti-I-J antisera on the growth of syngeneic methylcholanthrene-induced sarcomas S1509a and Sa-I, two tumors that have been shown to stimulate the development of specific suppressor T cells (1–3).

MATERIALS AND METHODS

Mice. A/J (H-2^a) female mice 8–10 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME). The hyperimmune mice used in the current experiments were 12–20 weeks of age.

Tumors. The S1509a tumor, a methylchloranthrene-induced sarcoma in A/J mice, was maintained both *in vitro* and *in vivo* by transfer of ascitic fluid obtained from the peritoneal cavity of mice given 10^5 sarcoma cells (1–3). Culture conditions have been described (1–3).

Tumor Sa-I was purchased from Jackson Laboratories and was maintained *in vitro* and *in vivo* in 3-month-old A/J mice in a similar manner to tumor S1509a (2). The Sa-I tumor was also maintained in culture in RPMI-1640 medium (Microbiological Associates, Bethesda, MD) supplemented with 5% fetal calf serum, penicillin, and streptomycin. Cells harvested after three *in vitro* subcultures were inoculated subcutaneously into normal A/J mice.

Procedure for Immunization. For immunization with the S1509a tumor, A/J mice received 10^6 cells subcutaneously in the back, and the tumor was removed surgically 1 week later. After several subsequent injections of live tumor cells (2), beginning 2 weeks after the initial excision of the tumor, the animals developed significant immunity to S1509a, as demonstrated by the rejection of an inoculum of 10^6 S1509a cells.

Antisera. The antisera used in these experiments included batch 480 of (3R × DBA/2)F_1 anti-5R, hereafter designated anti-I-J^k and prepared as described (8), which is capable of removing *I-*J^k-coded suppressor factors (8). As controls, batch 492 of (3R × 9R)F_1 anti-B10·HTT serum containing anti-I-J^s antibody (which does not crossreact with *I-*J^k-coded determinants) and normal A/J serum were used. In typical experiments, mice received daily intravenous injections of 0.2 ml containing 2 μl of neat serum. In other experiments, daily injections of 0.2 ml containing 20 μl of anti-I-J^k serum were administered. The quantity of antiserum used to reduce suppressor cell effects in the tumor systems was based on the independent demonstration that this amount of anti-I-J^k serum was effective in potentiating *in vivo* anti-SRBC antibody responses (12).

Tumor Assay. After tumor inoculation, mice were randomly assigned to experimental and control groups. Beginning on the third day after inoculation of tumors, animal hair was removed with a chemical depilating agent (Neet, Whitehall Lab, NY). The tumors were examined independently by two experimenters and tumor diameters were measured macroscopically with vernier calipers at right angles to calculate tumor size in cm^2.

Transfer of Suppressor Cells to Immune Mice. Splenocytes (3×10^7) from S1509a tumor-bearing mice were transferred intravenously into A/J mice immune to the tumor and these mice were rechallenged with 10^5 S1509a cells. Nonimmune A/J mice that received anti-I-J^k serum (at the time of injection of 10^6 S1509a cells and daily thereafter) served as donors of splenocytes for the experimental groups.

Statistical Analysis. The statistical significance of the results

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Abbreviations: SRBC, sheep erythrocytes; anti-I-J^k serum, serum containing antibodies against *I-*J^k gene-coded suppressor factors.

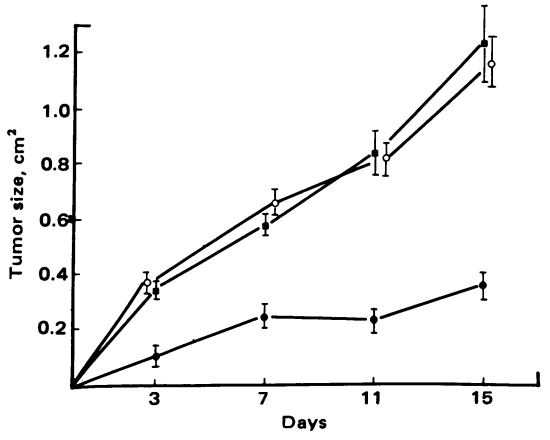

FIG. 1. The effect of anti-I-J serum on the growth of S1509a tumor in syngeneic mice. S1509a cells (10^5) were inoculated subcutaneously into A/J mice. One group of five mice (●) received anti-I-J^k serum at a dose of 2 μl/day per mouse. Control groups of five mice (○) received 2 μl/day per mouse of anti-I-J^s serum or Hanks' solution (■). Statistical significance, as determined by Student's *t* test, of the experimental anti-I-J^k serum-treated versus the Hanks' control group was $P < 0.01$ on day 3 and $P < 0.002$ on days 7, 11, 15. The anti-I-J^s serum-treated group and Hanks' control group did not differ significantly. Data shown as means ±SEM.

was calculated by using Student's *t* test as computed by the Wang programmable computer. Some experiments were performed "blind" by using coded antisera. The arithmetic means and standard errors of the means are indicated in the figures.

RESULTS

Effect of Anti-I-J^k Serum on the Primary Tumor-Bearing Host. It had been previously established (13) that intravenous administration of anti-thymocyte serum in doses of 100–200 μl/day to primary S1509a tumor-bearing mice could abrogate the enhancing effect of antigen-specific suppressor T cells. We examined the effect of 2-μl doses of anti-I-J^k serum administered *in vivo*, at the time of injection of 10^5-S1509a cells into nonimmune A/J mice and daily for 15 days thereafter. A control group received (3R × 9R)F_1 anti-B10·HTT serum, hereafter called anti-I-J^s serum, in quantities equal to those administered to the anti-I-J^k serum-treated groups. Another group of mice received Hanks' solution. Fig. 1 shows the ability of the anti-I-J^k serum to reduce tumor growth as early as day 3 after tumor inoculation. This effect persisted while the anti-I-J^k serum was administered (day 15 in this experiment). In contrast, the control anti-I-J^s serum had no discernible effect on the growth of the tumor. Independent experiments have established that the control serum contained comparable levels of anti-I-J^s activity as measured by the enhancement of the SRBC response of SJL mice.

Specificity of Anti-I-J^k Sera. In order to establish that the activity of anti-I-J^k serum was due to its capacity to interact with *I-J^k* gene products, the serum was absorbed with 4 × 10^8 B10·BR (H-2^k) lymphocytes or the same number of B10·D2 (H-2^d) lymphocytes. After absorption, the serum was administered daily beginning at the time of injection of 10^5 S1509a tumor cells. As can be seen in Fig. 2, all activity was lost after absorption of anti-I-J^k serum with H-2^k cells, whereas the serum absorbed on H-2^d cells still was capable of inhibiting tumor growth. It therefore can be concluded that the activity of the anti-I-J^k serum is based on its ability to interact with antigenic determinants coded by the *H-2^k* major histocompatibility complex and particularly by the *I-J^k* subregion and is not due to nonspecific contaminants in the serum.

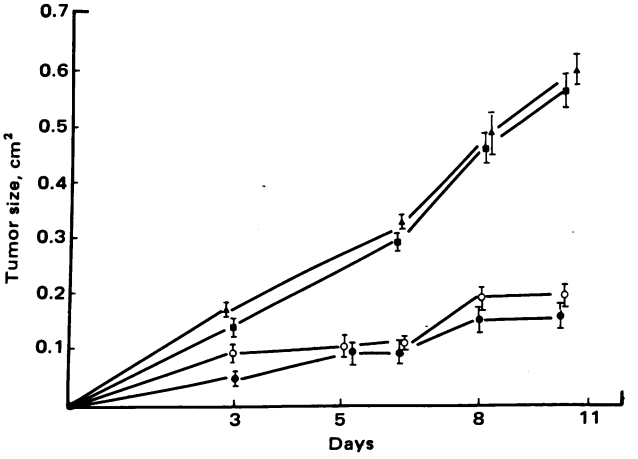

FIG. 2. The effect of absorbed anti-I-J^k serum on tumor growth. Untreated anti-I-J^k serum (2 μl/day per mouse) (○), either absorbed prior to use with 4 × 10^8 B10·BR (H-2^k) lymphocytes (▲) or 4 × 10^8 B10·D2 (H-2^d) negative control lymphocytes (●), was administered each day beginning at the time of inoculation of 10^5 S1509a cells into the respective groups of five A/J mice. A control group of five mice received Hanks' solution in lieu of antiserum each day (■). Statistical comparison of the groups with control revealed significance only with groups given either untreated serum or anti-I-J^k serum absorbed with B10·D2 cells ($P < 0.03$ on day 3 and $P < 0.002$ on days 5, 8, and 11). Data shown as means ±SEM.

Effect of Increased Amounts of Anti-I-J^k Antiserum. To assess whether larger amounts of anti-I-J^k serum could abolish tumor growth entirely, 20 μl of anti-I-J^k serum in 0.2 ml was administered to mice daily beginning at the time of injection of 10^5 S1509a tumor cells. As can be seen in Fig. 3, although 20 μl of the antiserum had a more dramatic effect on early tumor growth, by day 8 the effect was similar to that observed when the smaller dose was administered.

Effect of Anti-I-J^k Serum on a Larger Tumor Inoculum. We next investigated whether the anti-I-J^k serum could influence a larger tumor inoculum. To this end, 2 or 20 μl of anti-I-J^k serum was administered intravenously at the time of a 10^6 tumor cell inoculum and daily thereafter. Fig. 4 presents tumor growth curves in control animals and in animals treated with

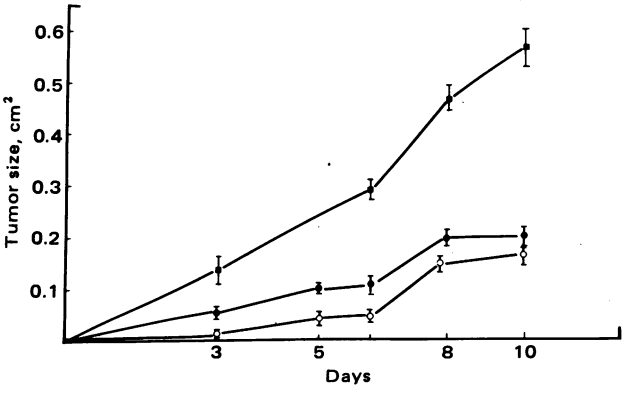

FIG. 3. The effect of larger doses of anti-I-J^k serum on S1509a tumor growth. Two (●) or 20 (○) μl of anti-I-J^k serum was administered daily beginning at the time of subcutaneous inoculation of 10^5 S1509a cells into groups of five mice. A control group received Hanks' solution on a daily basis (■) beginning with tumor implantation. Statistical comparison revealed that the group was receiving 20 μl of anti-I-J^k serum significantly different from the group receiving 2 μl only on day 3 ($P < 0.001$). Both anti-I-J^k serum-treated groups were highly significantly different from the control ($P < 0.002$) on days 5, 8, and 11. Data shown as means ±SEM.

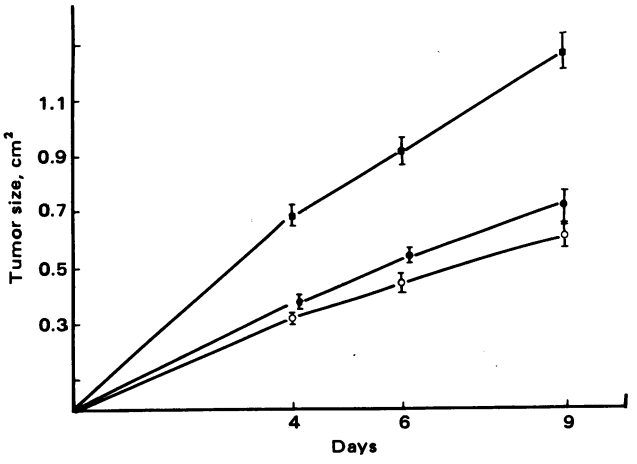

FIG. 4. Effect of anti-I-J^k serum on a large tumor inoculum. S1509a cells (10^6) were inoculated subcutaneously in the back of A/J mice. One group of five such mice received 2 μl of anti-I-J^k serum/day per mouse (●) and another group of five mice received 20 μl of anti-I-J^k serum daily per mouse (○), beginning at the time of tumor implantation. A control group of five A/J mice received Hanks' solution (■). Both the anti-I-J^k treated groups were highly significantly different from controls ($P < 0.005$) on days 4, 6, and 9. Data shown as means ±SEM.

anti-I-J^k serum daily beginning at the time of tumor implantation. Treatment with the anti-I-J^k serum clearly decreased the growth of a large tumor inoculum. There were no significant differences between the groups receiving daily doses of 2 or 20 μl of anti-I-J serum.

Effect of Anti-I-J^k on Suppressor Cells. Previous work has firmly established that adoptive transfer to immune mice of Thy-1-bearing lymphocytes obtained from tumor-bearing hosts caused specific inhibition of the rejection of a 10^6 tumor cell challenge. Therefore, in order to determine whether treatment with the anti-I-J^k serum caused the loss of suppressor cell activity, mice treated with anti-I-J^k serum and also given a tumor inoculum of 10^6 cells were used as donors of lymphocytes to be transferred to tumor-immune A/J mice. These mice were rechallenged with a 10^6 tumor cell inoculum. As shown in Fig. 5, treatment of the donor with anti-I-J^k serum resulted in the loss of suppressive effect in the recipient when 3×10^7 lymphocytes were transferred. The control serum did not interfere with the ability of tumor-induced suppressor cells to inhibit the rejection of the tumor in immune mice. We conclude that the observed decrease in tumor growth in nonimmune mice treated with anti-I-J^k serum may be attributed to the loss of endogenous suppressor cell activity, and that the mice that have received anti-I-J^k serum can no longer be used as a source of exogenous suppressor cells for adoptive transfer into immune mice.

Effect of Anti-I-J^k Serum on the Growth of Sa-I. We investigated whether the growth of another syngeneic tumor known to induce suppressor T cells (14) could also be affected by treatment with anti-I-J^k serum. Viable Sa-I tumor cells (10^5) were inoculated into nonimmune A/J mice that were then given 2 μl of anti-I-J^k serum daily. Fig. 6 shows that the growth of Sa-I was significantly inhibited by the administration of the antisuppressor serum.

DISCUSSION

In recent years, it has become evident that the gene products of the *I* region of the mouse *H-2* complex regulate specific immune responses to thymus-dependent antigens (15, 16). Specific *I* region immune response (*Ir*) and immune suppression (*Is*) genes control the capacity to form antibodies to a large number of synthetic and natural antigens and to develop specific suppressor T cells, respectively (17).

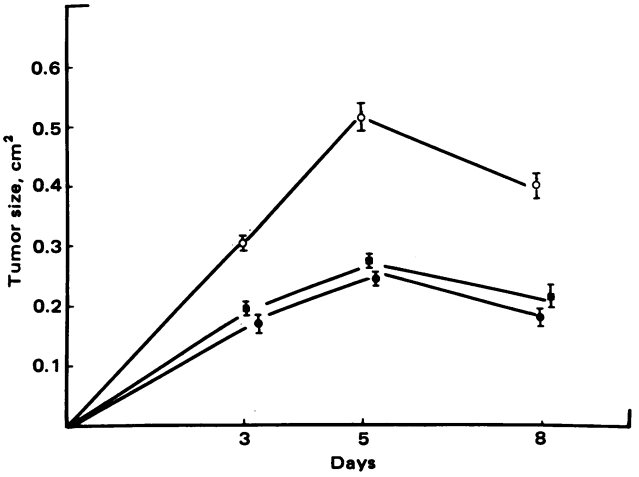

FIG. 5. The effect of anti-I-J^k serum on the transfer of S1509a-induced suppressor T cell. Groups of five A/J mice hyperimmune to S1509a cells received 10^6 S1509a cells subcutaneously with either 5×10^7 splenocytes obtained from A/J mice that had received 10^6 S1509a cells subcutaneously 7 days previously (○) or with 5×10^7 splenocytes obtained from A/J mice that had received both 10^6 S1509a cells per mouse 7 days earlier and also 2 μl of anti-I-J^k serum/day per mouse for that 7-day period (●). A control group of five hyperimmune mice received the same S1509a tumor cell challenge and Hanks' solution (■). The anti-I-J^k treated groups were highly significantly different from controls ($P < 0.005$) on days 4, 6, and 9. Data shown as means ±SEM.

Moreover, suppressor T cells (9) and suppressor factors (8, 10, 11) bear determinants coded for by the *I-J* subregion of the murine major histocompatibility complex.

Treatment of mice with microliter amounts of specific

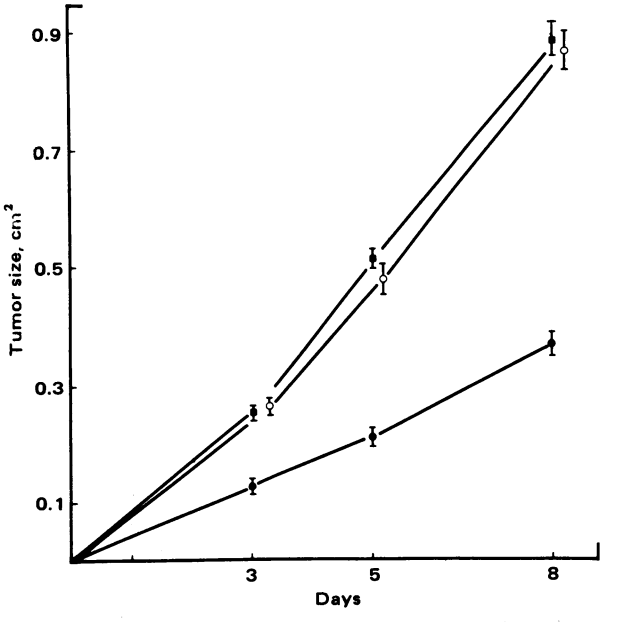

FIG. 6. The effect of anti-I-J^k serum on the growth of Sa-I. Groups of five to eight A/J mice received 10^5 Sa-I cells subcutaneously and also 2 μl of anti-I-J^k serum/day per mouse (●), or the same amount of normal A/J serum (○), or Hanks' solution (■). Statistical comparison of the anti-I-J^k serum-treated group with the control group was highly significant ($P < 0.002$) on days 3, 5, and 8. Data shown as means ±SEM.

anti-I-J alloantisera increases the primary antibody response to suboptimal challenge with SRBC (12). This adjuvant effect of anti-I-J antisera can be prevented by absorption with lymphoid cells bearing the appropriate I-J specificities and was therefore attributed to ability of the sera to interfere with the development of specific suppressor T cell activity (12).

In the present study, we investigated the effect of treatment with anti-I-J serum on the growth *in vivo* of two syngeneic fibrosarcomas, S1509a and Sa-I, known to stimulate the development of tumor-specific suppressor T cells. Microliter amounts of an antiserum directed against the I-J specificities of the tumor-bearing host prevented the rapid growth of these tumors that is normally observed in control mice or in mice treated with an antiserum specific for allogeneic I-J determinants. The microscopic appearance of representative tumors taken from anti-I-J serum-treated animals or controls was studied; histologic examination revealed clear differences between these groups. Anti-I-J serum-treated animals displayed marked mononuclear cell infiltrates into all levels of the tumor; great numbers of pyknotic and dead tumor cells were also seen in all sections. In sharp contrast, control tumors were infiltrated with inflammatory cells only to a slight extent, and this infiltration was limited to the margins of the tumor.

A most remarkable finding in these experiments and in the study of the adjuvant effect of anti-I-J serum on the response to SRBC is the marked *in vivo* activity of anti-I-J serum, as demonstrated by the exceedingly small amounts required to obtain maximal effects. The anti-I-J antibodies may exert their adjuvant effects on immune responses at such low concentration by several mechanisms. The antibodies may cause the opsonization of suppressor T cells bearing I-J specificities selectively because these cells constitute a relatively minor component of the peripheral T lymphocyte population. The anti-I-J antibodies may specifically bind suppressor factor and thereby interfere with its biological activity. Either of these hypotheses is consistent with the observation that treatment with anti-I-J serum effectively eliminated suppressor T cell activity in the spleen of S1509a tumor-bearing mice, at a time when tumor-bearing animals not treated with anti-I-J serum have in their spleen tumor-specific suppressor T cells capable of enhancing tumor growth adoptively in immune tumor-bearing recipients.

Highly significant suppression of the growth of S1509a tumor was observed after daily treatment with 2 μl of anti-I-J serum. The injection of a 10-fold larger amount of antiserum (20 μl) was somewhat more effective, particularly shortly after tumor inoculation, but did not result in the elimination of the tumors. The increased effect of a larger dose of anti-I-J serum, however, was transient and, after 2 weeks, the growth of the tumors was similar in the group treated with 2 or 20 μl of anti-I-J serum. These results suggest that in the tumor systems used in our experiments the development of suppressor T cells is an early event in the growth of a progressing tumor.

These results together with earlier experiments (1–3) have demonstrated the important role that tumor-specific suppressor T cells play in protecting antigenic tumors against the host immune defenses and have provided a novel approach to modifying the host–tumor relationship in favor of the host. The extent to which these results can apply to other experimental tumor systems should be carefully evaluated.

Moreover, the murine immune system has been an excellent model for the human immune system. The identification of distinctive alloantigens on mouse suppressor T cells (9) and suppressor factors (8, 10, 11) strongly suggests that distinctive antigens also exist on human suppressor T cells and suppressor factor. The considerable antisuppressive activity of anti-I-J serum in the mouse raises the expectation that an antiserum specific for the human counterpart of the mouse I-J determinants could have useful therapeutic properties.

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