CORRELATION OF SUPPRESSOR CELL DEVELOPMENT IN PARENTAL AND F₁ HYBRID MOUSE STRAINS WITH THE GROWTH OF A PARENTAL TUMOR IN VIVO

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Suppressor cells have been shown to be involved in the regulation of immune responses in genetic nonresponder animals (1, 2), as well as in those animals which have been tolerized to certain antigens (3, 4). Cells exhibiting suppressor activity have been recently identified in the spleens of tumor-bearing hosts (5, 6). These cells were able to nonspecifically suppress a variety of immune responses by normal syngeneic cells. It is not known whether this type of suppression observed in tumor-bearing animals involves immunological mechanisms such as those responsible in the antigen systems described above, or whether the suppression is due to overloading the immune system of the host with tumor cells.

The present report examines the roles of suppressor cells during host-tumor interaction in which the tumor is rejected, as well as that during a progressively growing lesion in an unresponsive mouse strain. Using a spontaneously derived AKR/J carcinoma-like tumor,¹ differential growth patterns were observed in the parental AKR/J, as compared to (AKR/J × DBA/2)F₁ and (AKR/J × C57BL/6)F₁ hybrids. This study demonstrates that depression of immune responsiveness and the generation of suppressor cells, appears to correlate with the strength or weakness of the specific anti-tumor responses in these strains of mice.

Materials and Methods

Mice. 6- to 8-wk-old male AKR/J, (AKR/J × DBA/2)F₁, and (AKR/J × C57BL/6)F₁ were purchased from The Jackson Laboratory, Bar Harbor, Maine, and housed in animal rooms at the Immunology Branch, NIH, Bethesda, Md.

Tumors. All animals were injected subcutaneously with 5 × 10⁶ AKR/J carcinoma-like tumor cells¹ in Hanks' balanced salt solution at a total vol of 0.25 ml. At 10–12 days postinjection, the size of the tumors in the AKR/J and AKB6F₁ were approximately equal (10–12 mm diameter), while that in the AKD2F₁ was somewhat larger (15–18 mm).

In Vitro Generation of Antibody Responses. Responding spleen cells were cultured for 3 or 4 days with trinitrophenyl-lipopolysaccharide (TNP-LPS) or sheep erythrocytes (SRBC), respectively, in 96-well flat bottom Microtest II plates (Falcon Plastics, Div. of BioQuest, Oxnard,

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¹ Levy, R. B., R. L. St. Pierre, A. D. Barker, and D. A. Axler. 1976. The characterization and relationship between in vivo and in vitro spontaneously arising neoplasms. Manuscript submitted for publication.
Specific plaque-forming cells (PFC) per $10^7$ cultured spleen cells were measured as previously described.\(^2\)

**In Vitro Generation of Cytotoxic Responses.** Responding spleen cells were incubated with irradiated (10,000 rads) syngeneic AKR/J tumor cells or 2,000 rads irradiated allogeneic spleen cells as described elsewhere.\(^2\) After 5 days of incubation, effector cells were harvested and assayed for specific cell-mediated cytotoxicity.

**Cytotoxic Assay.** Target cells, L5MF-22 (H-2\(^b\)) lymphoma, P815-Y (H-2\(^d\)) mastocytoma, and AKR/J (H-2\(^k\)) carcinoma-like cells were labeled with $^{51}$Cr and incubated at 37°C in 5% CO\(_2\) with effector cells for 4-5 h as described elsewhere.\(^3\) The percentage specific release of $^{51}$Cr was calculated as follows:

$$\frac{\text{experimental cpm} - \text{control cpm}}{\text{total cpm freeze-thaw} - \text{background cpm}} \times 100.$$  

**Results and Discussion**

Spleen cells from AKR/J, AKD2F\(_1\), and AKB6F\(_1\) mice were sensitized in vitro against AKR/J tumor cells. The results in Table I show that AKB6F\(_1\) and AKR/J, but not AKD2F\(_1\), spleen cells were able to generate specific anti-tumor effector cells. The secondary cell-mediated anti-tumor responses, in which spleen cells from mice preimmunized with $5 \times 10^6$ viable tumor cells were sensitized against the same tumor in culture, showed similar results.\(^3\) These in vitro findings correlated with the in vivo growth pattern of the tumor in these strains. Thus, the ADK2F\(_1\) hybrid, which did not reject the tumor in vivo, also appears to be unable to respond effectively against the tumor in vitro. The AKR/J and AKB6F\(_1\) animals, which generated a successful anti-tumor response in vivo, were also capable of effecting a significant cell-mediated anti-tumor response in vitro. Thus, the H-2\(^d\) allele appeared to be associated with susceptibility, whereas the H-2\(^b\) and H-2\(^k\) alleles were associated with resistance. It is noteworthy that the strongest anti-tumor responses were associated with the H-2\(^b\) allele,\(^3\) which has previously been found to be associated with strong resistance to murine leukemia virus-induced neoplasms.\(^7\)

The data in Table II illustrate that the splenic lymphocytes of tumor-bearing (12 day) AKR/J and AKD2F\(_1\) mice were markedly depressed in their ability to generate allogeneic effector cells after a primary in vitro sensitization against the relevant antigens. In contrast, the AKB6F\(_1\) spleen cells did not demonstrate any depression in their ability to elicit such a response. Mixtures of spleen cells from tumor-bearing mice of all three strains with normal syngeneic spleen cells failed to demonstrate any "true" suppressive activity, i.e., the decreased level of response in these cultures could be accounted for by a dilution effect of the unresponsive population (Table II). We have also found that depression of mixed lymphocyte and mitogen reactivity follows a similar pattern in these strains.\(^4\) Thus, the cellular immune reactivity in 12-day

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\(^2\) Levy, R. B., S. D. Waksal, R. L. St. Pierre, G. M. Shearer, and W. D. Terry. 1976. Immune suppression in tumor-bearing AKR/J mice. II. Mitogen responsiveness, mixed lymphocyte reactivity, and anti-SRBC responses of tumor-bearing mice during progressor and regressor stages of development. Manuscript submitted for publication.

\(^3\) Levy, R. B., S. D. Waksal, and G. M. Shearer. Control of immunity in parental F\(_1\) hybrid mouse strains to a spontaneously arising parental tumor. I. Correlation between in vivo and in vitro anti-tumor reactivity in responsive and unresponsive animals. Manuscripts in preparation.

\(^4\) Levy, R. B., S. D. Waksal, and G. M. Shearer. Control of immunity in parental and F\(_1\) hybrid mouse strains to a spontaneously arising parental tumor. II. Development of nonspecific suppressive activity in tumor-bearing mice. Manuscript in preparation.
posttumor injected AKR/J, as well as the unresponsive AKD2F1 mice, was found to be markedly depressed, but suppressive activity, capable of inhibiting these responses by normal populations, was not demonstrable.

Similar to the depression observed in the aforementioned responses, we also found (Fig. 1 A) that AKR/J and ADK2F1 spleen cells from 12-day tumor-bearing hosts were strikingly deficient in their ability to generate direct PFC's to both T-dependent (SRBC) and T-independent (TNP-LPS) antigens after primary in vitro sensitization. The highly responsive AKB6F1 strain again displayed no depression in its ability to produce PFC's to either of the two immunogens. When spleen cells from the AKR/J and ADK2F1 animals were mixed with their respective syngeneic normal spleen populations (Fig. 1 B), the direct PFC responses of these cultures to both SRBC and TNP-LPS were suppressed to levels similar to that of the unresponsive spleen cells. Therefore, unlike the cell-mediated response to allogeneic spleen cells, antibody responses elicited by spleen cells from normal mice could be suppressed by spleen cells from tumor-bearing AKR/J and AKD2F1 hosts.

There are several interpretations which could explain the finding that antibody responses, but not cell-mediated sensitization, could be suppressed by
spleen cells from tumor-bearing AKR/J and AKD2F1 mice. The suppressor population generated could be specific for antibody-forming cell precursors or helpers, but not effector cell precursor populations. Although suppressor cells in other systems (5, 6) have been reported to inhibit both types of responses, it was not conclusively determined whether a single population was responsible. The suppression of antibody production in tumor-bearing AKR/J mice could prevent the accumulation of blocking factors (8), thereby increasing the effect of the host's cell-mediated rejection processes. A second explanation could be attributable to a difference in the susceptibility to suppression of the two types of responses. This could mean that a qualitatively single population of suppressors is capable of suppressing both antibody- and cell-mediated responses, but that the latter becomes susceptible only when significant levels of these cells are generated. We have recently found4 that in the unresponsive AKD2F1 strain, suppressor cells which inhibit primary sensitizations of normal syngeneic spleen cells against alloantigens appeared during a period of tumor growth after that when cells able to suppress antibody responses were demonstrable.

Cantor et al. have recently shown that the generation of suppressive activity in response to erythrocyte antigens is directly proportional to the amount of immunizing antigen (3). Helper activity of spleen cells from hyperimmune animals was drastically lower than that of similar cells from optimally immu-
The level of antibody response may therefore depend on the proportion of T-helper and T-suppressor cells produced. Thus, a shift in favor of the latter, for example, might result in a state of overall unresponsiveness. Kapp et al. (1) and Benacerraf et al. (2) have shown that suppressor, but not helper, cells are generated to synthetic antigens in genetic nonresponder mice. Thus, these systems of antibody responses to antigen demonstrate that the generation of helper and/or suppressor cells depends upon both the dosage of antigen, as well as the genetic capability of the responding population. The same factors appear to be critical in the interaction of the host with tumor. After tumor injection, a highly responsive strain (AKB6F1) may develop only effector mechanisms, with a minimal, if any, degree of suppression. This would result in a successful (i.e., rejection) response. A less responsive strain (AKR/J), subjected to the identical tumor dose, may generate a greater degree of suppressive activity, and therefore approach the critical equilibrium between positive and negative factors which determines the overall success or failure of the response. An unresponsive strain (AKD2F1) may selectively produce only suppressors, resulting in progressive tumor growth. It is noteworthy that the number and/or strength of these cells were found to increase as the tumor developed in the AKD2F1 animals. Thus, it is possible that this strain is genetically incapable of generating effector cells in response to the tumor examined.

Such a schema would also suggest that all responders could be subjected to some critical tumor dosage, large enough to induce numbers of suppressors able to shift the equilibrium to a state resulting in irreversible tumor growth. This is analogous to the finding that large doses of immunizing antigen lead to the generation of suppressor cells responsible for the ensuing lack of specific antibody production (4). Such a critical dosage would vary for each tumor within each strain, and would decrease as the genetic capability of the responding host decreased. Maintaining the integrity of the organism by successful rejection of tumor may, therefore, depend on: (a) the genetic capabilities of the host, i.e. immune response genes which may control anti-tumor responses, as well as (b) the tumor burden, dependent on the site of growth and the proliferative capacity of the neoplasm.

**Summary**

Parental AKR/J, and AKB6F1, and AKD2F1, hybrid mice were injected subcutaneously with a spontaneously arising AKR/J tumor. The highly responsive AKB6F1 strain never exhibited any depression of immune functioning during the course of tumor growth and regression. The (AKR/J) intermediately responsive strain, while able to generate a successful anti-tumor response, did display a transient reduction of immunological capability, but only during the period of tumor growth and not during tumor regression. Cells able to suppress antibody, but not cell-mediated responses, were found. The unresponsive AKD2F1 strain was characterized by both a marked depression of immune responsiveness, as well as the generation of suppressor cells to both antibody, and later, cell-mediated responses. Depression of immune responsiveness, and the generation of suppressor cells, appeared to correlate with the strength or weakness of the anti-tumor response in these strains of mice.
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References

1. Kapp, J. A., C. W. Pierce, S. Schlossman, and B. Benacerraf. 1974. Genetic control of immune responses in vitro. II. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid$\alpha\alpha$-L-alanine$\beta\beta$-L-tyrosine$\gamma\gamma$ (GAT). J. Exp. Med. 140:648.

2. Benacerraf, B., J. A. Kapp, P. Debré, C. N. Pierce, and F. Dela Croix. 1975. The stimulation of specific suppressor T-cells in genetic non-responder mice by linear random co-polymer of L-amino acids. Transplant. Rev. 26:21.

3. Cantor, H., F. W. Shen, and E. A. Boyse. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. II. Activation by antigen: after immunization, antigen-specific suppressor and helper activities are mediated by distinct T-cell subclasses. J. Exp. Med. 143:1391.

4. Weitzman, S., and H. Cantor. 1976. Regulation of the immune response by T-cells in hyperimmune animals. Fed. Proc. 35:862.

5. Kirchner, H., T. M. Chused, R. B. Herberman, H. T. Holden, and D. H. Lavrin. 1974. Evidence of suppressor cell activity in spleens of mice bearing primary tumors induced by Maloney sarcoma virus. J. Exp. Med. 139:1473.

6. Gorczynski, R. 1974. Immunity to murine virus induced tumors. II. Suppression of T-cell mediated immunity by cells from progressor animals. J. Immunol. 112:1826.

7. Lilly, F., and T. Pincus. 1973. Genetic control of murine viral leukemogenesis. Adv. Cancer Res. 17:231.

8. Baldwin, R. W., M. R. Price, and R. A. Robbin. 1972. Blocking of cell-mediated cytotoxicity against rat hepatoma cells by tumor specific antigen-antibody complexes. Nat. New Biol. 238:185.