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Modulation of Growth of Malignant Cells by Anti-Idiotypic Immunity

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A. Introduction

One of the mechanisms capable of regulating an immune response involves the interaction of idiotypes and anti-idiotypes. In selected nontumor systems it has been shown that anti-idiotypic immunity can very specifically either suppress or stimulate immune responses (for review see Eichmann 1978). Thus, anti-idiotypic reagents may provide powerful tools for manipulating the induction or course of the host's immune responses to malignant cells.

To test this hypothesis, we have developed a tumor model in which anti-idiotypic immuni-

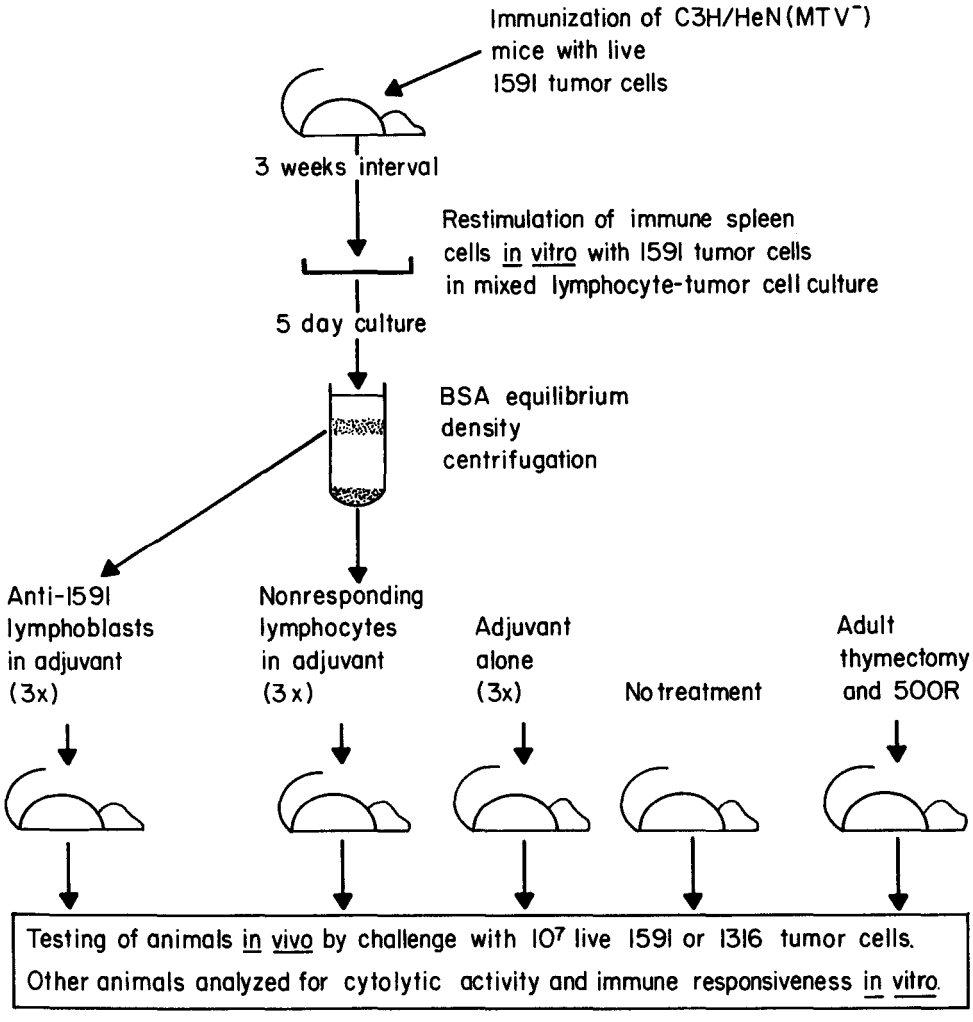

Fig. 1. Experimental protocol used for the generation and purification of tumor-specific lymphoblasts, immunization with these cells, and subsequent testing of blast-immunized and control animals. For details see Flood et al. (1980)

ty of mice to syngeneic tumor-specific lymphocytes can be elicited and analyzed (Flood et al. 1980). It had previously been shown that animals immunized with syngeneic purified antigen-specific lymphoblasts isolated from mixed lymphocyte cultures can produce anti-idiotypic immune responses to their own alloantigen reactive T cells (Andersson et al. 1976, 1977; Aguet et al. 1978; Binz and Wigzell 1978). We have adapted this system to syngeneic responses against tumor antigens and we can now manipulate the immune response in such a way that tumors will grow in animals which would normally reject these tumors.

B. Materials and Methods

The fibrosarcomas 1591 and 1316 induced by ultraviolet light in C3H/HeN (MTV$^-$) specific pathogen free mice were used (Kripke 1977). These tumors have non-cross-reactive tumor antigen and regularly regress upon transplantation into normal young syngeneic C3H mice (Fisher and Kripke 1977), but grow progressively in thymectomized x-irradiated mice. The general approach for induction of anti-idiotypic immunity by immunization with tumor-specific lymphocytes is shown in Fig. 1 and has previously been described in detail (Flood et al. 1980).

C. Results

We have found that immunization with tumor-specific lymphoblast induces unresponsiveness to 1591 tumor cells in vivo and in vitro (Fig. 2). Furthermore, we have found that immunization with 1591 specific lymphoblasts induced cytolytic anti-idiotypic T cells to these lymphoblasts. This was shown by an in vitro assay in which 1591 tumor-specific lymphoblasts were used as ^{51}-Cr-labeled target cells for the anti-idiotypic effector cells. Several lines of evidence suggested the specificity of the observed effects. Resistance of the 1591 blast-im-

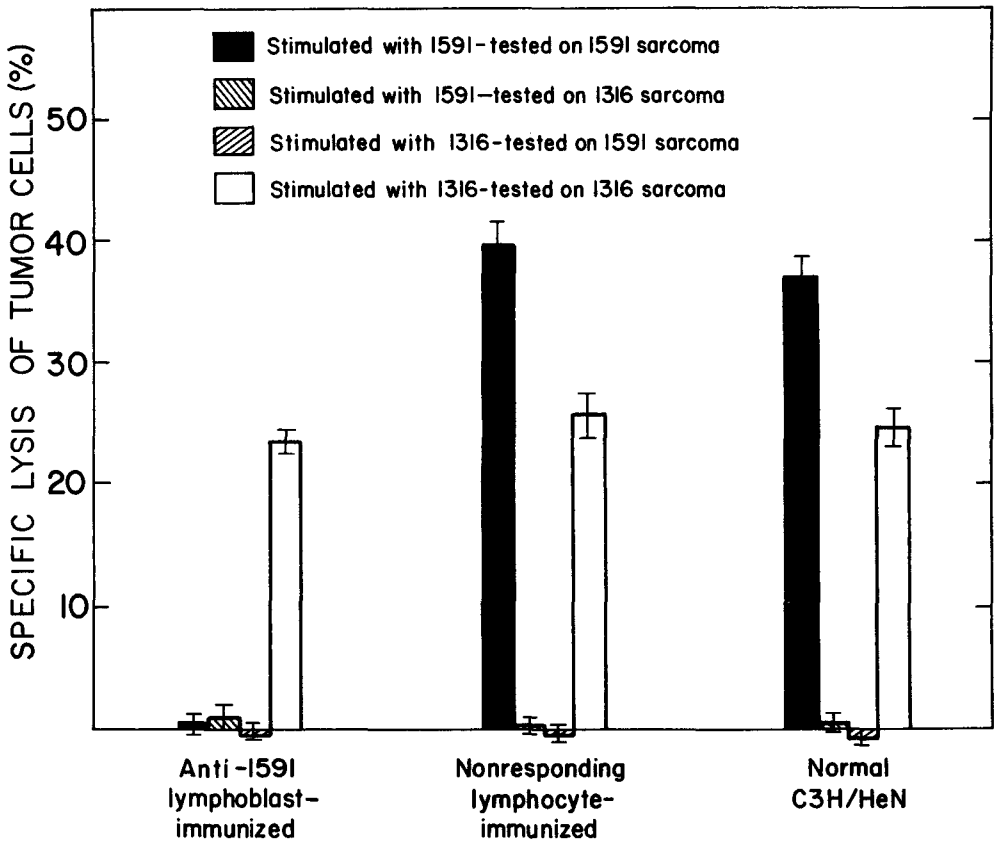

Fig. 2. Specific unresponsiveness of spleen cells from anti-1591 lymphoblast-immunized animals to 1591 tumor cells in culture. Spleen cells were stimulated in a 5 day primary mixed lymphocyte tumor cell culture with 1591 or 1316 tumor cells. The culture-generated effector cells were tested in a 3 h ^{51}Cr-release assay at a 100:1 effector to target cell ratio. Blast-immunized or control animals were tested 10 days after the third immunization

munized animals to 1316 tumor cells in vivo and responsiveness of the spleen cells from these animals in vitro were unimpaired. In agreement with this is the finding that spleen cells from the 1591 tumor-specific lymphoblast-immunized animals did not kill 1316 tumor-specific lymphoblasts. Furthermore, immunization with nonresponding lymphocytes or with lymphoblasts not having specificity for 1591 tumor cells were ineffective in inducing the observed effects.

Strong evidence indicating idiotype-specific immune reactions to tumor-specific lymphocytes came from the analysis of animals which responded to the tumor in spite of the blast immunization. These animals exhibited the same capability to specifically lyse 1591 tumor cells as control animals, yet they responded by generating 1591 specific lymphoblasts that were completely insensitive to lysis by anti-idiotypic effector cells (Table 1). This is in contrast to normal and control animals, which regularly responded by generating tumor-specific lymphocytes sensitive to the anti-idiotypic effector cells.

D. Discussion

The findings suggest that autologous idiotype-specific immunity against a tumor-specific lymphocyte clone, which is regularly present in

Table 1. Presence of a common 1591 specific idiotype on tumor-specific lymphocytes from normal and control animals which is absent in the tumor-specific lymphocytes of animals which broke idiotype-specific immune suppression

Source of target cells (anti-1591 lymphocytes)		Batch of anti-idiotypic effector cell probe (anti-anti-1591 lymphocytes)[a]	Specific lysis % of[b]	
Pretreatment	Animal no.		anti-1591 lymphoblasts	anti-1316 lymphoblasts
Normal animal	1	24-1	58	<0
	2	24-2	56	<0
	3	30-4	76	<0
	4	30-5	47	<0
	5	30-6	44	2
	6	30-7	53	<0
	7	31-1	36	<0
	8	31-2	33	N.D.
	9	35-1	44	<0
	10	35-1	50	<0
	11	35-1	56	<0
	12	35-1	54	<0
	13	35-1	44	<0
	14	35-1	43	<0
	15	44-2	56	<0
Nonresponding lymphocyte immunized animals	1	28-1	42	1
	2	28-2	54	7
	3	30-9	53	<0
	4	30-9	54	<0
Blast-immunized animals which broke suppression	1	28-1	0	1
	2	28-2	1	7
	3	30-9	1	<0
	4	30-9	0	<0

[a] Effector spleen cells were obtained from different batches of C_3H mice immunized three times with 10^7 1591-specific lymphoblasts in adjuvant

[b] Target cells were tested in a 4 h ^{51}Cr release assay using as target purified 1591-specific or 1316-specific lymphoblasts at a 250:1 effector-to-target cell ratio. These lymphoblasts were obtained after in vitro restimulation of spleen cells from normal, control, or blast-immunized tumor-responsive animals and purified by equilibrium density centrifugation

normal animals (Table 1), can be induced by the blast immunization. Thus, immunity eliminated the animals' normally predominant tumor-reactive lymphocyte clone, and these animals died of progressive tumor growth when challenged with the 1591 tumor cells, unless they were capable of breaking suppression with an idiotypically different, previously silent or undetected lymphocyte clone. The possible role of antigenic stimulation in the development of new clones (Cunningham and Pilarski 1974) is not clear in our system, but we have only observed the development of secondary 1591 specific clones in animals which have previously been blast immunized. Our experiments give strong evidence for the importance of tumor-specific lymphocytes in the primary defense of the onimmune host to malignant cells and raise the possibility that natural killer cells may not play a decisive role in the defense of C3H mice against these tumors. Interestingly, older mice or ultraviolet light irradiated young mice which are susceptible to the 1591 and 1316 tumors cannot develop 1591 specific lymphocytes in vivo or in vitro, while they do develop natural killer activity (manuscript in preparation). It will be interesting to determine whether anti-idiotypic reagents, used under defined conditions, may stimulate tumor-specific lymphocyte clones in such animals and thereby induce in these mice resistance to the malignant cells.

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