Malignant growth in the normal host after variant selection *in vitro* with cytolytic T-cell lines

(immunosurveillance/unique tumor-specific antigens/somatic cell variants)

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Communicated by A. A. Moscona, December 19, 1983

ABSTRACT Using variant selection *in vitro*, we have explored the changes necessary to convert potentially malignant cells into progressively growing cancer cells. As a model, we studied the murine ultraviolet light (UV)-induced tumor 1591-RE, which routinely regresses in the normal syngeneic host. The antigenicity of this tumor is specified by multiple independent tumor-specific antigens, named A, B, C, and D. Cells of this tumor were exposed *in vitro* to cytolytic T-cell lines specific for these separate antigens. Tumor variants were isolated that displayed selective antigenic losses. By challenging normal syngeneic mice with the variants, we could determine the significance of each antigen in tumor rejection—i.e., a switch from a regressor to a progressor phenotype upon selective loss of certain antigens. We found that variants which lost the A and C antigens grew progressively in normal mice, whereas variants which had lost the B antigen were still rejected like the parental tumor cells. Furthermore, the B and D antigens could be expressed on progressively growing variants. Thus, loss of the A and C antigens, but not the B and D antigens, was required for allowing malignant growth. Selection for loss of one T-cell-recognized antigen never selected simultaneously for loss of any of the other T-cell-recognized antigens, nor did such selections affect sensitivity to activated macrophages or natural killer cells. Therefore, our results strongly suggest that loss of defined components of a complex T-cell-recognized antigen are sufficient to allow escape from immunosurveillance.

Certain tumors induced by physical or chemical carcinogens are very effectively rejected by the normal syngeneic host. Excellent examples of such immunosurveillance mechanisms (1) are observed with ultraviolet light (UV)-induced tumors in mice (2) and apparently also in man (3). Experimentally, it has been shown that normal mice are completely resistant to challenge with even extremely large doses of these UV-induced tumor cells. However, as in other tumor systems, these tumors can escape the immune defenses of the normal immunocompetent host, and the escaped tumors regularly consist of phenotypically altered variants (4-8). Such host-selected changes represent the "fingerprints" of the normal immune selection pressures, and study of these fingerprints can give some insight into the complexity of different immune defense mechanisms operating in the normal host. But obviously only those changes for which a probe is available can be identified, so that in fact the number of phenotypic changes resulting from host selection may be much greater than discovered. Furthermore, several of the observed changes may merely be coincidental and not essential for allowing tumor escape.

In this paper, we have taken, therefore, a different approach to study the phenotypic changes involved in allowing potentially malignant cells to grow progressively in a normal

host. This was done by selecting *in vitro* with cytolytic T-cell lines for tumor variants that displayed defined antigenic changes and then testing the growth behavior of these variants *in vivo*. The results demonstrate that loss of certain T cell-recognized unique tumor-specific antigens was sufficient for converting the potentially malignant cells into cancer cells that grow progressively in normal mice; loss of sensitivity to natural killer (NK) cells or activated macrophages (M ϕ) was not required for this conversion.

MATERIALS AND METHODS

Animals and Tumor Lines. Five- to 10-week-old female C3H/HeN mammary tumor virus-negative mice were purchased from the National Cancer Institute Frederick Cancer Research Facility and maintained as described (4). The syngeneic fibrosarcomas 1591-RE and 2240-RE were induced by UV irradiation, have noncrossreacting tumor-specific transplantation antigens, and spontaneously regress when transplanted into normal syngeneic mice (2, 4, 8-10). 1591-RE1 and 1591-RE2 are two cloned tissue culture cell lines of the 1591-RE tumor that were independently adapted from solid tumor fragments of the fifth or first transplant generations, respectively. All the above tumors can be serially transplanted in nude mice (2). 1591-M ϕ .1 is a variant of 1591-RE2 selected *in vitro* for resistance to activated M ϕ (8). The above cell lines were cultured in minimal essential medium containing 10% heat-inactivated fetal bovine serum and penicillin at 100 units/ml, and streptomycin at 0.1 mg/ml as described (4). The Moloney virus-induced murine lymphoma YAC was maintained *in vitro* in supplemented RPMI 1640 medium (11).

Variant Selection *in Vitro*. Generation and maintenance of the anti-A, anti-B, and anti-C T-cell lines are described elsewhere (9, 12). Antigen-loss tumor variants were selected *in vitro* by exposing tumor cells to the cytolytic T-cell lines, using methods previously described (12). All selections were made with the cloned anti-A, the uncloned anti-B, or the uncloned anti-C T-cell lines.

Generation of NK Cells and Activated M ϕ . NK cells were generated as described (13). Briefly, spleen cells from syngeneic normal mice injected 24 hr earlier with 0.1 mg of poly(inosinic acid)-poly(cytidylic acid) [poly(I-C); Sigma catalog no. P 4136] in saline intraperitoneally were dispersed by grinding through a wire screen, washed with minimal essential medium/fetal bovine serum/penicillin/streptomycin, and used as effector cells. Activated M ϕ were generated as previously described (8).

Chromium Release Assay. The cytotoxicity of cells from 7-day-old passage cultures or NK cells was determined by us-

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Abbreviations: AS, variants selected for resistance to the anti-A T-cell line; BS, variants selected for resistance to the anti-B T-cell line; CS, variants selected for resistance to the anti-C T-cell line; Isu, lytic susceptibility units; M ϕ , macrophage(s); NK, natural killer; poly(I-C), poly(inosinic acid)-poly(cytidylic acid); RE, regressor.

ing a method described elsewhere (9). The percentage of specific lysis was calculated by the formula:

$$\% \text{ specific lysis} = [(\text{experimental release} - \text{spontaneous release}) / (\text{total release} - \text{spontaneous release})] \times 100.$$

Spontaneous release was <15% of the total release of radio-label. The cytotoxicity of activated M ϕ was determined in a 16-hr release assay as described (8). Lytic susceptibility units (lsu) were used to compare the relative lytic susceptibilities of different target cell lines to a given effector cell, in analogy to the use of lytic units that have been previously described (14, 15) to quantitate differences in the lytic activity of different effector cells to a given target cell. lsu values were calculated from individual dose-response curves by taking the reciprocal of the fraction of 10^6 effector cells that caused specific lysis of 50% of given target cells.

RESULTS

Loss of the A, B, or D Antigens and Its Effect on Malignant Growth Behavior in Normal Hosts. 1591-RE1 and 1591-RE2 are two cloned tissue culture cell lines of the 1591-RE tumor and regress when transplanted into normal syngeneic mice

(4). 1591-RE1 expresses all of the four presently defined 1591-RE-specific antigens (A, B, C, and D), while 1591-RE2 expresses only the A and B antigens (9). Because of its lack of the C and D antigens, 1591-RE2 appeared more suitable for determining the individual contribution of the A and B antigens in tumor rejection. Thus, variants of 1591-RE2 were selected *in vitro* with the anti-A or anti-B cytolytic T-cell lines for loss of the A or B antigen (called AS or BS variants, respectively). Fig. 1 shows the antigenic phenotypes of six AS variants (*Upper*) and six BS variants (*Lower*) as defined by their susceptibility to lysis by the anti-A, anti-B, or anti-C T-cell lines in a ^{51}Cr release assay. The antigenic expression of these variants was compared to that of the 1591-RE1 and 1591-RE2 tumors as positive controls and to the unrelated UV-induced 2240-RE tumor as a negative control. Apparently all of the six AS variants had specifically lost the A antigen yet retained the B antigen. Thus, the loss of the A antigen was selective. Interestingly, however, reactivity with the anti-C T-cell line was observed with one of the six AS variants (AS2), although it was not detected with the parental tumor 1591-RE2. The unexpected reactivity of the AS2 variant with the anti-C T-cell line will be discussed below. The six BS variants all lost the B antigen, while the A antigen was retained. Thus, again the loss of the appropriate antigen was selective. Furthermore, the C antigen did not appear on any of these variants after selection.

We next tested the growth behavior *in vivo* of these antigen-loss variants of 1591-RE2 that specifically lost either the A or B antigens. The tissue culture cells were first grown to a solid tumor in congenic nude mice, and then 5- to 10-week-old normal syngeneic mice were challenged subcutaneously in each inguinal area with two 2-mm 3 fragments of the solid

FIG. 1. Antigenic phenotype of variants selected from the parental regressor tumor line (1591-RE2) for loss of the A antigen (AS variants; *Upper*) or the B antigen (BS variants; *Lower*) with the appropriate T-cell line. The variants and control targets were tested in a 4.5-hr ^{51}Cr release assay for sensitivity to the cytolytic anti-A, anti-B, and anti-C T-cell lines. Data are not shown for the expression of the A and B antigens on 1591-RE1.

Table 1. 1591 variants selected *in vitro* for loss of the A antigen can grow progressively in the normal host

Derivation	Designation	Phenotype*				Tumor incidence [†]	
		A	B	C	D	Nos.	%
Parental	1591-RE2	+	+	-	-	1/300	0
Anti-A selected	AS1	-	+	-	+	7/7	100
	AS2	-	+	+	+	0/7	0
	AS3	-	+	-	+	5/7	71
	AS4	-	+	-	+	7/7	100
	AS5	-	+	-	+	7/7	100
	AS6	-	+	-	-	0/7	0
Anti-B selected	BS1	+	-	-	-	0/5	0
	BS2	+	-	-	ND	0/5	0
	BS3	+	-	-	ND	0/7	0
	BS4	+	-	-	ND	0/5	0
	BS5	+	-	-	ND	0/5	0
	BS6	+	-	-	ND	0/5	0
Randomly selected	SUB1	+	+	-	ND	0/7	0
	SUB2	+	+	-	ND	0/7	0
	SUB3	+	+	-	ND	0/6	0
	SUB4	+	+	-	ND	0/6	0
	SUB5	+	ND	-	ND	0/7	0
	SUB6	+	ND	-	ND	0/7	0
	SUB7	+	ND	-	ND	0/7	0
	SUB8	+	ND	-	ND	0/7	0
	SUB9	+	ND	-	ND	0/7	0
	SUB10	+	ND	-	ND	0/7	0

ND, not done.

*Expression of the A, B, and C antigens for several of these tumor cells is shown in Fig. 1. Data are not shown for expression of the D antigen.

[†]Number of mice with progressively growing tumors 6 weeks after tumor challenge/number challenged. The percentage of mice affected is also tabulated. All animals that had tumors at 6 weeks eventually died because of the progressive tumor growth.

tumor. This protocol ensured that the *in vitro*-selected tumor variants had not lost the capability of growing progressively *in vivo* in the immunodeficient host. Table 1 shows that while the parental 1591-RE2 tumor regularly regressed, four out of six of the AS variants grew progressively. In contrast, selection for loss of the B antigen did not select for variants capable of malignant growth in normal hosts, since all of the six BS variants regressed when transplanted to normal mice. Similarly, all ten random clones of 1591-RE2 (SUB1 to SUB10) were rejected by the normal mice challenged. The AS variants were also tested for expression of the D antigen. Although the D antigen appeared to be absent from the parental 1591-RE2 tumor, we found that five of the six AS variants reacted with the anti-D T-cell line with a susceptibility similar to the 1591-RE1 tumor ($A^+ B^+ C^+ D^+$) (data not shown). Apparently the expression of the B and D antigens did not prevent malignant growth, since these antigens were expressed on the four AS variants that progressed in normal mice.

Loss of the C Antigen and Its Effect on Malignant Growth Behavior in Normal Hosts. Although all of the AS variants had lost the A antigen, two out of six of these variants still regressed in normal mice. It was therefore important to determine if other antigens were present on these two variants that were responsible for their regressor phenotype. We found that one of these two variants, AS2, expressed the C antigen ($A^- B^+ C^+ D^+$), and this additional antigen conceivably could have contributed to the rejection of this variant. We therefore attempted to select for variants of an $A^- B^+ C^+ D^+$ regressor tumor with the anti-C T-cell line for loss of the C antigen. Unfortunately, we have not been able to isolate such C^- variants from the AS2 cells; apparently the frequency of such spontaneous variants was still too low in this population, which had been cloned and continuously passaged for 2 months. We have, however, been successful in selecting for loss of the C antigen from a different $A^- B^+ C^+ D^+$ variant, called AS7. Like AS2, AS7 was derived from the 1591-RE2 tumor and regressed in normal mice. However, AS7 had been selected for loss of the A antigen with a monoclonal antibody and a fluorescence-activated cell sorter rather than by selection with the A antigen-specific T-cell line. This syngeneic monoclonal antibody has a reactivity pattern identical to the anti-A T-cell line as determined by reactivity of both probes with a panel of UV-induced tumors as well as antigen-loss variants of the 1591-RE tumor selected with either the antibody or the anti-A T-cell line (unpublished results). Fig. 2 *Upper* shows that every variant of AS7 selected with the anti-C T-cell line had lost the C antigen and retained the B antigen. Unexpectedly, one of the four selected variants (AS7.CS4) expressed the A antigen, although this antigen had not been detectable in the parental AS7 population. Variants of AS7 were also selected with the anti-B T-cell line for loss of the B antigen. Fig. 2 *Lower* shows that these AS7.BS variants had lost the B antigen, retained the C antigen, and did not express the A antigen. Thus, we had selected variants of AS7 that had specifically lost either the B or C antigens.

Table 2 shows the growth behavior of these variants in normal syngeneic mice. Evidently three of the four AS7.CS variants showed increased progressive growth in normal mice. In contrast, both variants of AS7 that lost the B antigen (AS7.BS variants) were completely regressive. Thus, the expression of the C antigen, like that of the A antigen, was always associated with rejection, while its absence could allow tumor progression unless other regressor antigens were still expressed.

Sensitivity of Antigen-Loss Variants to NK Cells and Activated $M\phi$. We next determined if selection for T-cell-resistant variants concomitantly selected for a decreased sensitivity to other effector cells that can kill neoplastic cells.

FIG. 2. Antigenic phenotype of variants selected from the 1591-AS7 regressor cell line for loss of the C antigen (AS7.CS variants; *Upper*) or the B antigen (AS7.BS variants; *Lower*) with the appropriate T-cell line. The indicated targets were tested in a 4.5-hr ^{51}Cr release assay for sensitivity to the cytolytic anti-A, anti-B, and anti-C T-cell lines. Data are not shown for the expression of the B and C antigens on 1591-RE1.

Thus, the sensitivity to poly(I-C)-induced Lyt-2-negative cytolytic spleen cells (NK cells) was determined for 1591-RE2, AS variants, BS variants, and random clones of 1591-RE2 by using these tumor cells as targets in a ^{51}Cr release assay. Fig. 3 shows that each of these different groups of tumor cells displayed a similar NK sensitivity, which was distinctly lower than that of YAC cells. To compare quantitatively these NK sensitivities, Isu were determined for each target cell as

Table 2. 1591 variants selected for loss of the C antigen can grow progressively in the normal host

Derivation	Designation	Phenotype*				Tumor incidence†	
		A	B	C	D	Nos.	%
Parental	AS7	-	+	+	+	0/7	0
Anti-C selected	AS7.CS1	-	+	-	ND	5/6	83
	AS7.CS2	-	+	-	ND	6/6	100
	AS7.CS3	-	+	-	+	3/18	17
	AS7.CS4	+	+	-	ND	0/6	0
Anti-B selected	AS7.BS1	-	-	+	ND	0/6	0
	AS7.BS2	-	-	+	+	0/6	0

ND, not done.

*Expression of the A, B, and C antigens on these tumors is shown in Fig. 2. Data are not shown for expression of the D antigen.

†See Table 1.

FIG. 3. Sensitivity of subclones of 1591-RE (*Left*) and variants selected for loss of the A or B antigens (AS and BS variants respectively; *Right*) to NK cells. Spleen cells from mice injected intraperitoneally 24 hr earlier with 0.1 mg of poly (I-C) were used as effectors in a 5.5-hr ^{51}Cr release assay against the indicated targets.

described in *Materials and Methods*, and the mean lsu values of each group were compared (results are expressed as mean $\pm$ SEM). Such analysis demonstrated that the NK sensitivity of the AS variants (2.5 ± 0.7 lsu), BS variants (1.8 ± 0.3 lsu), and random clones of 1591-RE2 (2.3 ± 0.6 lsu) were statistically indistinguishable (in all cases $P > 0.6$). In addition, comparison of the NK sensitivity of the AS variants that grew progressively (3.0 ± 1.5 lsu) to those that regressed (2.0 ± 0.3 lsu) was also not statistically different ($P > 0.5$).

The sensitivity of the AS variants and the parental 1591-RE2 to activated M ϕ was also determined. Fig. 4 shows that the parental 1591-RE2 was lysed by the activated M ϕ , whereas a M ϕ -resistant variant of 1591-RE2 (1591-M ϕ -1)

FIG. 4. Sensitivity of variants selected from 1591-RE2 for loss of the A antigen (AS variants) to activated macrophages. Adherent peritoneal exudate cells from mice injected intraperitoneally 5 days earlier with Brewer's thioglycolate were cultured for 5 hr with lipopolysaccharide and secondary mixed lymphocyte culture supernatant as described (8) and used as effectors in a 16-hr ^{51}Cr release assay against the indicated targets. The results of four separate experiments were similar and therefore pooled; vertical bars indicate the SEM of the results for individual experiments.

was resistant to lysis. The M ϕ sensitivities of the AS progressor and regressor variants were similar to the sensitivity of the parental 1591-RE2. To compare quantitatively these M ϕ sensitivities, lsu values were determined for each target. When the mean lsu values for lysis of the AS progressor (3.3 ± 0.8 lsu) and regressor (4.2 ± 1.9 lsu) variants, and the parental 1591-RE2 (2.5 lsu) were compared, all of these groups were found to be statistically indistinguishable (in all cases $P > 0.6$). Thus, antigen-loss variants that grew progressively were unaltered in their sensitivity to NK cells and activated M ϕ .

DISCUSSION

We have found that the selective loss of certain components of a T-cell-recognized unique tumor-specific antigen was sufficient to allow the potentially malignant 1591-RE tumor to grow progressively in normal syngeneic mice. Antigen-loss variants of a cloned 1591-RE cell line were selected *in vitro* with cytolytic T-cell lines specific for each of the independent antigenic components of the 1591-RE tumor; these 1591-RE-specific antigens are called A, B, C, and D (9). By studying the growth behavior of such variants *in vivo*, we hoped to detect the "single-step" antigenic changes required for tumor progression. We found that the A and C but not the B and D antigens were critical for tumor rejection. This is consistent with the previous finding that host-selected progressor variants of 1591-RE lost the A antigen and retained the B antigen (12). Several findings indicate that the antigen-loss variants expressed well-defined antigenic changes. Each T cell line only selected for loss of the appropriate antigen and never for loss of the other T-cell tumor-specific antigens recognized (Figs. 1 and 2) or for loss of other cell surface components such as H-2 K or D (9). Furthermore, the T-cell-selected variants were not significantly altered in their sensitivity to activated M ϕ or NK cells (Figs. 3 and 4). This is in contrast to host-selected progressor variants of the 1591-RE tumor that not only lost T-cell-recognized antigens but also demonstrated increased resistance to activated M ϕ (8). Apparently, a selection process was required to isolate progressor variants, because such variants were not detected in random clones of the parental tumor cell line. In addition, the selection process was specific, since variants selected for loss of the B antigen still displayed a regressive phenotype as long as the A or C components were expressed. We do not know of other investigations that have converted potentially malignant cells into cancer cells by antigenic selection *in vitro*. Interestingly,

however, Maryanski *et al.* (16) have studied the antigenicity of a tumor that had become highly immunogenic after mutagenization of the tumor *in vitro*. They found that such artificially introduced antigenicity was composed of two independent components, and the results suggested that selection *in vitro* for loss of one of these components was associated with increased malignant growth.

Why were two of the six AS variants and one of the four AS7.CS variants as regressive as their parental populations? One explanation is that these variants regressed because they expressed still other rejection antigens. For example, in the case of AS2 ($A^- B^+ C^+ D^+$) and AS7.CS4 ($A^+ B^+ C^- D^+$) it seems likely that the C and A antigens, respectively, were responsible for rejection. The reason for the rejection of AS6 ($A^- B^+ C^- D^-$) is less clear, however, since this variant does not express either of the two 1591-RE antigens (A and C) associated with a regressive phenotype. One possibility is that AS6 expresses other rejection antigens that are yet undefined. Such antigens may in fact be recognized by immune cells other than cytolytic T cells (e.g., DTH or help-inducing T cells) and therefore have not yet been detected by our approach for acquiring tumor-specific probes. Subsets other than cytolytic T cells have been implied to be of primary importance in allograft and tumor rejection (17, 18), although their relative importance is still uncertain (19). We also cannot exclude the possibility that such a hypothetical rejection antigen is often lost from the parental tumor along with the A and C antigens, but in the case of AS6 it remained behind. Several other explanations for the rejection of AS6 appear less likely. For example, although AS6 expressed the B antigen, this regressor variant did not induce a stronger anti-B response, nor did it have more B antigen on its surface than AS progressor variants that also express the B antigen (unpublished results). Another possibility is that AS6 was less capable of growing *in vivo* due to nonimmunologic mechanisms (e.g., slower growth rate, decreased elaboration of angiogenesis factor, etc.). This is unlikely, however, since AS6 grew as well as the progressor variants in congenic nude mice (unpublished results). Therefore, it appears that some immunologic mechanism is responsible for the rejection of AS6 in normal syngeneic mice and that AS6 has retained its sensitivity to immune effector cells other than tumor-specific cytolytic T cells. However, in this study we show that AS6 is not altered in its sensitivity to two likely candidates for such effector cells, NK cells and activated $M\phi$. Further analysis of the host response to AS6 will be useful for elucidating the mechanisms responsible for rejection of 1591-RE.

In several instances, tumor variants unexpectedly expressed antigens that were undetectable in the parental population from which the variants were selected. For example, AS2 (C^+) was selected from 1591-RE2 (C^-) and AS7.CS4 (A^+) was selected from AS7 (A^-). Similarly, the D antigen was expressed on variants (AS1, AS2, AS3, AS4, and AS5) selected from 1591-RE2 (D^-). The appearance of these antigens was particularly surprising since we have shown that the antigen loss variants are phenotypically stable during continuous passage in culture or repeated passage in congenic nude mice for up to 4 mo (9). Possibly, the newly expressed antigens were absent from the populations from which the variants were selected and their appearance was induced by the selection process, or possibly, these newly expressed antigens were preexistent on a small undetectable percentage of the parental population. We also do not know if the antigens that reappeared are even identical to the original antigenic molecules since their "reappearance" is solely

defined by reactivity with the particular 1591-RE-specific T-cell line. At present, we cannot distinguish between any of these possibilities, since (i) a biochemical characterization of the antigens is lacking, (ii) frozen cells of the parental 1591-RE2 line immediately after the original cloning are not available and, (iii) AS7 was not cloned before further selection. It is apparent that the expression of "new" determinants will have to be examined carefully, since such a finding may have important implications for understanding the mechanism of antigenic variation of tumor cells.

Clearly the evolutionary progression of a normal cell to a cancer cell is a multistep process (20) that involves a series of heritable phenotypic alterations leading to independence from many kinds of homeostatic control and defense mechanisms of the host (21). To dissect this multistep process it would obviously be important to isolate variants showing defined single-step alterations (22). Thus, the variants described here help to map one of the changes in the pathway that transformed cells must go through before they can become malignant tumors in a normal host.

We thank Dr. Donald A. Rowley for a critical review of this manuscript. We also thank Ms. Connie Philipps and Ms. Karen Schmidt for excellent technical assistance. This study was supported by National Cancer Institute Grants RO1-CA-22677 and PO1-CA-19266. H.S. was supported by RCDA CA-00432, and R.D.W. and J.L.U. by National Institute of General Medical Sciences Grant PHS-T32-GM-07281.

1. Ehrlich, P. (1909) *Ned. Tijdschr. Geneesk.* **5**, 273-289.
2. Kripke, M. L. (1977) *Cancer Res.* **37**, 1395-1399.
3. Hardie, I. R., Strong, R. W., Hartley, L. C. J., Woodruff, P. W., & Clunie, G. J. A. (1980) *Surgery* **87**, 177-183.
4. Urban, J. L., Burton, R. C., Holland, J. M., Kripke, M. L., & Schreiber, H. (1982) *J. Exp. Med.* **155**, 557-573.
5. Uyttenhove, C., Maryanski, J., & Boon, T. (1983) *J. Exp. Med.* **157**, 1040-1052.
6. Bosslet, K., & Schirmacher, V. (1981) *J. Exp. Med.* **154**, 557-562.
7. Weinhold, K. J., Miller, D. A., & Wheelock, E. (1979) *J. Exp. Med.* **149**, 745-757.
8. Urban, J. L., & Schreiber, H. (1983) *J. Exp. Med.* **157**, 642-656.
9. Wortzel, R. D., Philipps, C., & Schreiber, H. (1983) *Nature (London)* **304**, 165-167.
10. Urban, J. L., Holland, M., Kripke, M. L., & Schreiber, H. (1982) *J. Exp. Med.* **156**, 1025-1041.
11. Burton, R. C., & Winn, H. J. (1981) *J. Immunol.* **126**, 1985-1989.
12. Wortzel, R. D., Urban, J. L., Philipps, C., Fitch, F. W., & Schreiber, H. (1983) *J. Immunol.* **130**, 2461-2466.
13. Djeu, J. Y., Heinbaugh, J. A., Holden, H. T., & Herberman, R. B. (1979) *J. Immunol.* **122**, 175-181.
14. Cerottini, J.-C., Engers, H. D., MacDonald, H. R., & Brunner, K. T. (1974) *J. Exp. Med.* **140**, 703-717.
15. Lutz, C. T., & Fitch, F. W. (1979) *J. Immunol.* **122**, 2598-2604.
16. Maryanski, J. L., & Boon, T. (1982) *Eur. J. Immunol.* **12**, 406-412.
17. Loveland, B. E., Hogarth, P. M., Ceredig, R., & McKenzie, I. F. C. (1981) *J. Exp. Med.* **153**, 1044-1057.
18. Bhan, A. K., Perry, L. L., Cantor, H., McCluskey, R. T., Benacerraf, B., & Greene, M. I. (1981) *Am. J. Pathol.* **102**, 20-27.
19. Mills, C. D., & North, R. J. (1983) *J. Exp. Med.* **157**, 1448-1460.
20. Foulds, L. (1954) *Cancer Res.* **14**, 327-339.
21. Nowell, P. C. (1976) *Science* **194**, 23-28.
22. Collins, J. L., Patek, P. Q., & Cohn, M. (1980) *Contemp. Top. Immunobiol.* **11**, 1-79.