Immunobiology of the Tumor-Host Relationship

edited by
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PREFACE

Just 15 years have elapsed since the work of Richmond Prehn, George Klein, and Lloyd Old, among others, undermined the long-standing concept of the tumor–host relationship as autonomous tumor growth in a supplicative and supportive host. This old concept still provides the rationale for most therapeutic approaches to cancer today. Gradually, it has been replaced by a much more dynamic and biologically sound set of ideas involving active and specific resistance on the part of the host, tightly interlocked with other functions of the lymphoreticular system.

The supporting data-base is still distressingly small and restricted in content directly relevant to tumor biology. However, it has generated a conviction on the part of many investigators that full understanding of the immunobiology of the tumor–host relationship provides the most logical and sanguine of all current approaches to many unsolved problems of cancer. This conviction was shared by most, but not all of our colleagues who gathered in Milan to cope with the implications of the rapidly growing mass of new and pertinent data. The goal was to assess, to integrate, and to synthesize these data as testable hypotheses, and to evaluate them as potentially applicable to clinical problems in man.

The central paradox of the tumor–host relationship from the point of view of the tumor biologist is still embodied in the unrelenting, lethal growth of primary malignant neoplasms, despite amply documented evidence of a specific, strong, and multicomponent immunologic response to the tumor. The conferees in Milan can be said to have failed in their mission, since this paradox certainly has not been resolved, at least not in any terms which prescribe novel immunotherapeutic approaches in man. On the other hand, they did succeed in bringing to light for the first time several important and relevant lines of evidence which further define the dynamics of the tumor–host relationship. They also fabricated, in discussion of these data and by agreement on interpretations, some new and likely useful conceptual lattices on which to build future efforts.

Among such developments, several warrant emphasis here. Evidence came from many sources indicating that the uncomplicated idea of a single tumor-specific antigen is no longer viable. Immunogenic tumor-borne structures are probably large in number, and can include components usually defined as "self," including at the least enzymes, alloantigens, differentiation, and embryonic structures. The suggestion was that the immune response to such tumor antigens is not irrelevant to the relationship, even though such structures alone do not seem to provide targets in transplantation tests. Tumor antigens were in fact shown to be a

controlling element in the relationship, either acting alone or complexed with antibody. As targets for immunologic attack on tumor cells, these structures appear much more capricious than previously thought. These structures may respond to antibody-mediated attack, for example, by aggregating on the cell surface ("capping"), or by exocytosis ("shedding") into their environment as antigen–antibody complexes having potent biologic effects on attacking lymphoid cells. In either case this can leave the tumor cell functionally naked ("modulated") in terms of providing a proper attack point for potentially cytotoxic cells or antibody. Moreover, the first evidence was presented that this process can actually occur *in vivo*, both in terms of capping and shedding.

Much attention was given to the effector systems potentially capable of specific tumor cell destruction. Here it became quite clear that T lymphocytes are not the only "killer" cells, but that both B cells and other antibody-armed lymphoid cells share this property. Macrophages were shown to have even more power and significance in this role both *in vitro* and *in vivo* than previously envisioned. But macrophage involvement in tumor destruction has a paradoxical quality. Tumors were described that grow and kill the host, despite containing in their intracellular matrices up to 50% of macrophages. Moreover, evidence was considered that restricts the mechanisms involved in the general depression of delayed-type hypersensitivity observed in the tumor-bearing host to effector system malfunction, probably involving macrophages, rather than the T-lymphocyte deficit previously postulated.

The concept of immune surveillance emerged battered from the siege, but usefully transformed after a thoroughgoing reexamination. Crucial here was evidence for specific immunological effects involving cells or antibody, which stimulate or support tumor growth. Also germane were data demonstrating that the putatively T-deficient nude mouse is not without immune defenses to tumor growth. This provided but one element suggesting that a significant and important change in emphasis may be occurring in the direction of work of those involved in immunobiology. A gradual shift is perceived, from a preoccupation with experiments that employ tumor systems to explore basic immunobiologic problems, to studies which are primarily relevant to tumor biology. This conference, and the volume to which it has given birth, hopefully will give further impetus to this new direction.

RICHARD T. SMITH

MAURICE LANDY

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And to Ms. Linda Mannooch, Dept. of Pathology, University of Florida, the editors and the conferees express their gratitude for the help she extended in the preconference planning phase and in the preparation of the manuscript for publication.

CONTENTS

SESSION I

TUMOR ANTIGEN AS THE CONTROLLING ELEMENT IN THE TUMOR–HOST RELATIONSHIP

Multiplicity of tumor antigens and their relationship to self-components—F_1 Parental interaction in the MLC—Cross reacting versus specific tumor neoantigens—Alloantigens and embryonic structures as tumor antigens—Production and shedding of tumor antigens—Evidence for capping *in vivo*—Relationship between shedding, capping, and modulation—Occurrence of non-immunogenic tumors.

CHAIRMAN SMITH: The tumor-bearing host is perfused continuously with tumor membrane proteins, fragments, and even whole cells (reviewed in Smith, *New Eng. J. Med.*, **287**, 439, 1972). This antigenic barrage initially engages specific recognition receptors, leading to proliferation of lymphoid cell subsets, secretion of antibodies, and generation of cytotoxic cells. The intensity of the immune responses generated is such as to be indicative of hyperimmunity directed toward the various tumor membrane structures. This hyperimmune state is unique in that it is usually concurrent with the continuous circulation of the tumor membrane antigens which generates it. Paradoxically it is usually impotent in destruction of the established primary autochthonous tumor which gave it birth.

In beginning this session, I shall focus upon the nature of the tumor membrane structures, their possible sources, production, and distribution. Most of these structures are not of viral origin; thus, most are properly categorized as expressions of "self," and the responses they elicit arise from "self-recognition."

Table 1 concerns the heterogeneity of membrane structures presented by tumors. Solid evidence supports the concept that single tumor cells may also express, in their membrane, structures of direct or indirect viral origin. Any of the so-called "self-components"—those associated with embryonic development, organ or tissue specific differentiation structures, structures present at specified phases of the cell cycle, or structures novel because they represent degraded membrane products—may be immunogenic to the lymphoreticular system. Boyse (in *Immune Surveillance*, Smith and Landy, eds., p. 5, Academic Press, N.Y., 1970) has postulated that another rich source of unique structures may result from alloantigen matrix concatenations in the cell membrane, although no direct experimental evidence for this is known.

It should be obvious that most of the membrane structures comprising this are those usually defined as "self." Some other rather precise gene-locus products—structures determining histoincompatibility with respect to allogeneic hosts—are another possibility of polymorphic structures on tumors potentially

TABLE 1
Some Sources of Individual Membrane Structures
Potentially Stimulating to Cell Recognition Systems

Virion – tumor-associated or passenger virus
Virus directed or indirect structures
Differentiation directing or fetal structures
Cell cycle specialization structures
Cell membrane degradation products
Matrix or grid concatenations (Boyse)
Allotypic structures – universal or organ representation

TABLE 2
Specificity of F_1 Hybrid Subsets
Stimulated by Parental Cells in One-Way MLC

Source of reacting cells	Source of 1° mitomycin-blocked target cells	Source of 2° mitomycin-blocked target cells	^{3}H-thymidine incorporation (mean CPM ± SE)
(C57BL/6 × CBA)F_1	–	–	376 ± 42
(C57BL/6 × CBA)F_1	(C57BL/6 × CBA)F_1	(C57BL/6 × CBA)F_1	210 ± 18
(C57BL/6 × CBA)F_1	CBA	CBA	408 ± 74
(C57BL/6 × CBA)F_1	CBA	C57BL/6	3025 ± 218
(C57BL/6 × CBA)F_1	C57BL/6	C57BL/6	645 ± 87
(C57BL/6 × CBA)F_1	C57BL/6	CBA	2097 ± 109

affecting the tumor-host interaction. Three lines of investigation in our laboratory have led us to focus upon such antigens as a source of tumor-associated antigens, giving rise to (1) self-reacting clones, (2) antibodies, and (3) competing, perhaps suppressive, elements in tumor-host relationships.

Classical approaches to tumor immunobiology have centered on the elimination of residual heterozygosity in order to define TSTA through use of highly inbred animals. I should first like to show how heterozygosity may conceivably be a self-generated source of immunologically significant membrane structures in the tumor-bearing host. The critical element would be to demonstrate self-recognition clones for H-2–linked membrane structures in the mouse. In observations made over the past five years in our laboratory, and confirmed in several others, F_1 hybrid mouse lymphocytes, whether from thymus, spleen, or lymph node, were shown to proliferate significantly in one-way MLC with parental peripheral lymphocytes (Table 2). Recognition, signified by such proliferation, has all the characteristics we have defined for allogeneic cell combinations: alloantigen specificity, probable T dependency, augmentation by tumor-bearing, and B-cell triggering. Through "suicide"-type experiments, Gebhardt *et al.* (*J. Exp. Med.*, in press) have shown that different subsets or clones of cells in the F_1 hybrid spleen or lymph nodes proliferate in response to each parental target cell. Work in congenic lines further defines the stimulating structures on parental cells as being products of, or determined by, the MHC locus or by genes closely linked to it. The data do not disclose whether the stimulatory structure is LD-like, SD-like, the product of a latent virus-activating gene, an Ir gene product (Ia-1 locus?) or some unknown product of the H-2 locus. The data imply also that the recognition receptors on responding cells are not expressed codominantly as are SD locus products. Interpreted literally, the data signify that F, recognition of parent is clonal. Further, they infer that lymphocyte recognition subsets or clones, detectable only in the unique circumstances of *in vitro* culture of a coherent heterozygote, an F_1 hybrid between congenic

strains, may possibly also exist for similar "self"-membrane structures in outbred or noncoherent heterozygotes. Such self-recognition subsets must obviously be under effective steady-state control *in vivo*—"blocked," defused, or tolerant—thus prevented in some way from proliferating or expressing cytotoxicity. They are detectable only *in vitro* and in those circumstances in which the alloantigen recognition subset augmenting effect of tumor-bearing stimulates their proliferation, as we shall discuss later (Session V, pp. 309–310). Caution is urged in accepting without reservation this conceptual fallout of the data described because the phenomenon is limited to mouse cells at present; Wilson could find no evidence for the effect in PBL of rats. (Darcy Wilson, personal communication.)

A second line of evidence for elements of the histocompatibility complex having significant contributions to the tumor-host relationship is derived from experiments in which novel antigens are detected on tumor cells by anti-SD sera raised against normal cells. Lymphoblastoid cell lines derived from healthy human donors (HuLCL) and from infectious mononucleosis (IM) patients have been tested for cytotoxicity with a variety of supposedly monospecific anti-HL-A sera (Tables 3 and 4). Multiple positive reactions are found in the lines for determinants not represented in the donor's phenotype. Some, but not all, of these reactions are absorbed by donor cells. Moreover, in a series of over 50 congenic MCA tumors developed in our laboratory, Klein has found that multiple H-2 specificities, both "public" and "private," are detected by oligospecific anti-H-2 determinant antisera but are not represented in the phenotype detected in normal cells of the donor strain (Table 5). Absorption studies suggest that these, too, are not absorbed by normal cells. Other explanations of the phenomena are conceivable, we readily concede. These findings are, however, consistent with the hypothesis that some of the multiple membrane structures on the tumor cell are immunogenic components of normal cells but only readily detectable when expressed in tumor cells.

The third element in evidence for an immunologic activity toward histo-

TABLE 3
Comparison of Cytotoxicity Patterns of Anti-HL-A Typing Sera on Three LCL from a Single Donor

	Putative specificity of sera													
	1	2	3	9	10	11	4C	5	7	8	W10	12	13	4A/12
Donor				+		+	⊞					+		+
Line – 7A	⊞		⊞	+	+	+	⊞		⊞	+		+	⊞	+
– 7B				+	+		⊞		⊞	+		+	⊞	+
– 7C		⊞		+	+	+	⊞			+		+	⊞	+

TABLE 4
Cytotoxicity Comparison of Anti-HL-A Typing Sera on LCL and Respective LCL Donors

Donor	Line	Concurrent cytotoxicity	Disparate positives	
			Donor	Cell line
1.	B-6	19	0	9
2.	B-7	20	0 (1±)	7
3.	B-8	21	0	7
4.	B-9	13	0 (1±)	15
5.	B-10	24	0 (1±)	3
6.	B-11	10	0 (2±)	9

TABLE 5
Comparison of Cytotoxicity Patterns of Anti-H-2 Sera on Congenic MCA Tumors

Cells tested	Putative H-2 specificity of sera								
	15	52,53	23	31,34	33,53,54	8	11,25,54	16,34,35,41	4
C57BL/10·spleen		+			+		+	+	
C57BL/10·M2 tumor	+	+		+	+	+		+	+
C57BL/10·A·spleen		+	+			+	+	+	+
C57BL/10·A·M1 tumor	⊞			+	+	⊞		+	⊞

compatibility structures involved in tumor-bearing is the confirmation, in our laboratory, of Haywood and McKhann's (*J. Exp. Med.*, **133,** 1171, 1971) experiments showing inverse correlation between the detectability of H-2 specificities on tumor cell membranes and tumor immunogenicity. We found that the stimulating capacity of soluble tumor antigens has roughly an inverse relationship to the immunogenicity of the tumor from which it was obtained.

If membrane structures we have come to know as "histocompatible" antigens contribute in a major way to the tumor-host immunologic interaction, an obvious corollary is that "self" and "non-self" no longer carry the significance enshrined by our immunologic gurus. Self-tolerance would be a necessary, normal characteristic of a fragile, balanced system, a balance disturbed by the exceptional demands for disposal of membrane components, presented to the host by injury or by tumor bearing.

Next, I want to turn to a consideration of our concept of cross-reacting TSTA as being wholly different from TSTA or tumor-unique antigens. The original experiments establishing TSTA demonstrated that chemically induced tumors have a unique transplantation antigen, whereas virus-induced tumors cross react regardless of the organ system. Studies of the MTV system by our colleague Vaage indicated that some tumors have both types of antigenicity.

TABLE 6
Apparent *in Vitro* Cross Reactions among MCA Mouse Tumors[a]

LNC source by immunizing tumor	Colony inhibition of target tumor (%)	
	49	81
32	26.1	0
33	26.1	45.3
35	19.4	
44	11.6	
47		5.5
49	18.4-89.5 (4)	
51	19.4	0
53	33.0	
61	25.5	16.8
67		18.8
74	45.8	
81		26.5-91.8 (6)

[a]Calculated from data of Hellström, I, *et al.*, 1968.

TABLE 7
In Vitro Cytotoxicity of Congenic Anti-MCA Tumor Sera

Serum (1-5)	Target cell (C.I.)	
	B10·M2	B10·A·M1
B10 anti-B10·M1	0.75	0.66
B10 anti-B10·M2	0.51	0.35
B10·A anti-B10·A·M2	0.60	0.27
B10·BR anti-B10·BR·M1	0.57	0.47
B10·BR anti-B10·BR·M2	0.58	0.41

As *in vitro* assays are used extensively in studying chemically induced tumors, it becomes clear that whether tested by colony inhibition (Table 6), cell, antibody-mediated cytotoxicity (Table 7) or, as I shall describe, by stimulation assays for solubilized membrane structures, cross reactions are nearly universally encountered between chemically induced tumors.

These cross reactions are usually interpreted to signify that common or *shared* antigens exist in addition to the unique TSTA disclosed by transplantation tests. Hellström's data, shown in Table 6, are exemplary in this context.

In order to analyze so-called cross reactions in greater depth, Forbes, Nakao, Blackstock, and I have tested responses of various lymphoid cell subpopulations to soluble antigens from about 50 tumors, grown in 10 strains of mice (*Fed. Proc.*, **32**, 1020, 1973; manuscripts in preparation). We compared proliferative responses of spleen, PBL, and/or lymph node cells to a wide dosage

range of KCl-solubilized tumor and normal cell membranes, at varying intervals after tumor inoculation. Evidence which I will summarize, is interpreted to mean that different subsets of lymphoid cells are responding to *multiple* stimulatory components of the tumor membrane rather than a single large homogeneous subset responding to a single or a few shared antigens. Moreover, recognition subsets responsive to membrane structures present on normal muscle cells are stimulated and proliferate in the later period of tumor bearing in most animals.

When a typical stimulation pattern for peripheral blood cells (Fig. 1) or lymph-node cells (Fig. 2) is expressed as incorporation per 10^6 cells, an interesting effect is observed. Proliferating nodes or spleen show high unstimulated incorporation and this high level is inhibited—reduced toward control background levels, with *very* low amounts of the antigen—on the order of 0.1–0.5 μl/culture. Stimulation supervenes as more antigen is added, and peak stimulatory values are usually in the 10-50 μl/culture range. While the mechanism of inhibition is not understood as yet, it is tumor specific and appears to be mediated by a relatively low molecular weight component of the antigenic mixture. Based upon considerations detailed elsewhere (Smith and Konda, *Int. J. Cancer*, **12**, 577, 1973; Konda, Nakao and Smith, *Cancer Res.*, **33**, 2247, 1973; Konda and Smith, *Cell. Immunol.*, in press) expression of data per 10^6 cells fails to account for the occurrence of major changes in cell mass. Expression for cell mass *per se*, provides a more meaningful basis for comparisons (Fig. 3) between normal or nonregional node masses which have variable total cell numbers.

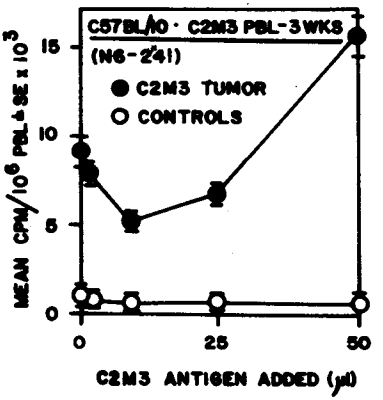

Fig. 1. Stimulation of ^{3}H-TdR incorporation of PBL, taken from C57BL/10·A mice, bearing C57BL/10·A C2M1 tumors of two weeks duration, by varying amounts of a KCl-solubilized C57BL/10 C2M1 tumor membrane preparation. Data are expressed as cmp/10^6 PBL ± SE. These and data in Figs. 2, 3, 4, 5, and 6 are taken from Forbes, Nakao, and Smith (manuscript in preparation).

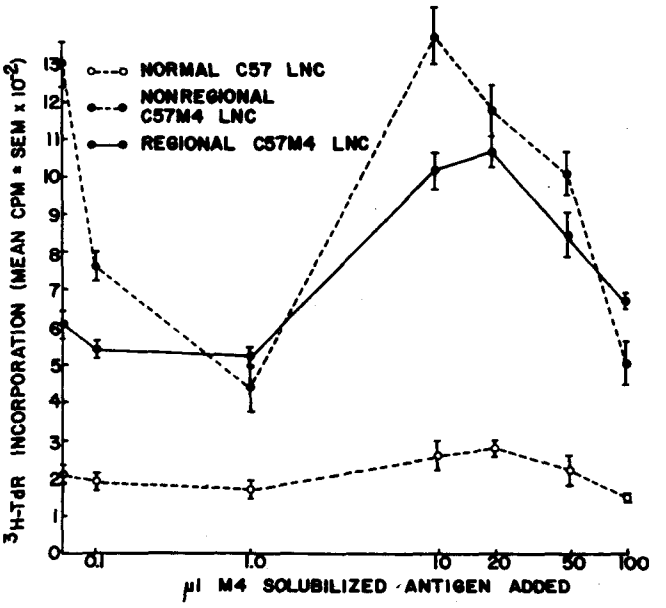

Fig. 2. C57BL/6 regional lymph node cell response to KCl solubilized C57BL/6-M4 antigens, 22 days after inoculation of 1×10^5 C57BL/6 M4 tumor cells.

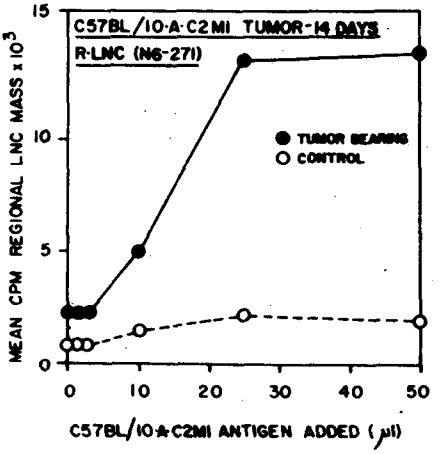

Fig. 3. Dose-response relationship between amount of C57BL/10 · A tumor antigen and ^{3}HTdR incorporation by regional LNC taken from mice bearing that tumor for 14 days. Data are expressed as cpm/R-LNC mass, as compared to effect of antigen on normal LNC.