Current Topics in Microbiology and Immunology

110

The Molecular Biology of Adenoviruses 2

Edited by Walter Doerfler

The Molecular Biology of Adenoviruses 2

30 Years of Adenovirus Research 1953–1983

Edited by Walter Doerfler

With 49 Figures

Springer-Verlag
Berlin Heidelberg NewYork Tokyo 1984

Professor Dr. WALTER DOERFLER
Institut für Genetik
der Universität zu Köln
Weyertal 121
D-5000 Köln 41

ISBN 3-540-13127-2 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-13127-2 Springer-Verlag New York Heidelberg Berlin Tokyo

Library of Congress Catalog Card Number 15-12910
Printed in Germany.

Typesetting, printing and bookbinding:
Universitätsdruckerei H. Stürtz AG, Würzburg
2123/3130-543210

Current Topics in Microbiology and Immunology

110

List of Contributors

BERNARDS, R., Department of Medical Chemistry, Sylvius Laboratories, Wassenaarseweg 72, NL-2333 AL Leiden

COOK, J.L., Department of Medicine, National Jewish Hospital and Research Center, Denver, CD 80206, USA

FIELD, J., Departments of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, USA

FRIEFELD, B.R., Departments of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

FUJINAGA, K., Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College, S-1, W-17, Chuo-ku, Sapporo 060, Japan

GALIBERT, F., Laboratory of Experimental Hematology, Centre Hayem, Hôpital St-Louis, F-Paris

GRONOSTAJSKI, R.M., Departments of Developmental Biology and Cancer, Albert Einstein, College of Medicine, Bronx, NY 10461, USA

GUGGENHEIMER, R.A., Departments of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, USA

HORWITZ, M.S., Departments of Cell Biology, Microbiology and Immunology, Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461, USA

HURWITZ, J., Departments of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, USA

KREVOLIN, M.D., Departments of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

LEVINE, A.J., State University of New York at Stony Brook, School of Medicine, Department of Microbiology, Stony Brook, NY 11794, USA

LEWIS, A.M., Jr., Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, USA

LICHY, J.H., Departments of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, USA

NAGATA, K., Departments of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, USA

SHIMIZU, Y., Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College S-1, W-17, Chuo-ku, Sapporo 060, Japan

TIKCHONENKO, T.I., D.I. Ivanovsky, Institute of Virology, 16, Gamaleya Street, 123098 Moscow, USSR

VAN DER EB, A.J., Department of Medical Biochemistry, Sylvius Laboratories, Wassenaarseweg 72, NL-2333 AL Leiden

VAN ORMONDT, H., Department of Medical Biochemistry, University of Leiden, NL-2333 AL Leiden

WADELL, G., Department of Virology, University of Umeå, S-901 85 Umeå

YAMASHITA, T., Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College S-1, W-17, Sapporo 060, Japan

YOSHIDA, K., Department of Molecular Biology, Cancer Research Institute, Sapporo Medical College S-1, W-17, Sapporo 060, Japan

Table of Contents

Indexed in Current Contents

The Interface Between Adenovirus-Transformed Cells and Cellular Immune Response in the Challenged Host

A.M. LEWIS, JR.[1] and J.L. COOK[2]

1 Introduction

The discovery by TRENTIN et al. (1962) that human adenoviruses were capable of producing tumors when inoculated into hamsters created a major role for these agents in the field of viral oncology. As participants in this field, several of the adenovirus (Ad) serotypes are among the most thoroughly studied animal viruses. One of the primary objectives of the study of these agents as tumor viruses has been to elucidate the mechanisms that are associated with their capacity to convert normal cells to neoplastic cells that produce tumors in animals. In approaching this objective, theoretical and technical developments have focused current research on the structure, organization, and expression of the Ad genome, and much has been accomplished. The functional arrangement of the Ad2 genome has been determined and the DNA sequence structure of several Ad serotypes is far advanced. The processing of Ad RNA into cytoplasmic mRNA that is translated into viral proteins has provided new insights into the mechanisms of RNA transcription in eukaryotic organisms. The mode of replication of the Ad genomes is under intensive investigation. The regions of the viral genome that are associated with the conversion of normal cells to neoplastic cells have been located, and many of the proteins encoded by these genes have been identified and in some cases purified. For detailed discussions of these developments, we refer the reader to other chapters in this volume and to recent reviews by FLINT (1980a, b), PERSSON and PHILIPSON (1982),

1 Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205, USA
2 Department of Medicine, National Jewish Hospital and Research Center, Denver, CO 80206, USA

Current Topics in Microbiology and Immunology, Vol. 110

CHALLBERG and KELLY (1982), and DOERFLER (1982). In spite of these impressive accomplishments, the goal of defining the mechanism of Ad-induced carcinogenesis has remained elusive, and it is becoming increasingly apparent that the question of how viruses and neoplastic cells produce tumors in animals will most likely remain after understanding of the molecular mechanisms of cell transformation (as defined by the induction of immortality) in vitro has been reached. The complexities of the interactions between cells rendered neoplastic by adenoviruses and the cellular immune defenses of the potential animal host suggest that new concepts and approaches to the possible mechanism of viral carcinogenesis are needed.

As tumors develop only in intact animal hosts, the cumulative studies of tumor-host relationships lead us to believe that the interactions at the interface between the incipient tumor and the cellular immune system of the potential host are the critical elements in the success or failure in the events leading to tumor development. To define these interactions and to develop the conceptual framework and the biological assays that will be essential for the success of a more refined molecular approach to the problems of tumor induction, the systematic study of the potential for tumor development in a number of animal models by oncogenic and nononcogenic Ad serotypes and the cells they transform will be necessary. In this chapter we will consider those phenomena that appear to be associated with the capacity of adenoviruses and Ad-transformed cells to induce the formation of tumors in animal hosts. To accomplish this objective, we will direct our remarks to the studies of Ad2, the most thoroughly studied nononcogenic Ad serotype, and Ad12, the most thoroughly studied oncogenic Ad serotype.

2 Patterns of Ad2- and Ad12-Induced Neoplasia In Vivo and In Vitro

Neoplasia as a biological entity is poorly understood. Basic concepts of the process have been derived from clinical and pathological studies of patients with neoplastic diseases. During the past half century, these clinicopathological observations, coupled with studies of tumor development in animals inoculated with oncogenic viruses or treated with chemical carcinogens, have provided a plausible sequence of events that appears to outline the conversion of normal cells to tumor cells in vivo (FOULDS 1969). The steps in this neoplastic conversion comprise an initiating event followed by a series of progressions from a more organized abnormal or atypical growth through a less organized hyperplastic or premalignant type of growth to invasive, metastasizing tumors. However, foci of tumor cells can appear within these lesions at any of the stages of development. To unravel the components of this process as it occurs in vivo, models are needed that mimic the various events in the conversion of normal cells to tumor cells.

Particularly relevant to the study of the sequential conversion of normal cells to tumor cells is the range of neoplastic capacities represented among

the various subgroups of human adenoviruses. Based upon the differences in their ability to induce tumors when inoculated into newborn Syrian hamsters (*Mesocricetus auratus*), adenoviruses have been classified into the highly oncogenic human subgroup A (serotypes 12, 18, 31); the weakly oncogenic subgroup B (serotypes 3, 7, 11, 14, 16, 21, 34, 35); and the nononcogenic subgroups C (serotypes 1, 2, 5, 6) and D (serotypes 8–10, 13, 15, 17, 19, 20, 22–30, 32, 33). HUEBNER et al. (1962, 1963b) found that hamsters carrying tumors induced by Ad12 and Ad18 developed antibodies that reacted by complement fixation to early nonvirion T (tumor) antigens. These antibodies were subsequently found to be subgroup specific, and their reactivity by the complement fixation test was used to further characterize the highly oncogenic, weakly oncogenic, and nononcogenic subgroups (HUEBNER 1967; GILDEN et al. 1968; MCALLISTER et al. 1969). The classification of human adenoviruses according to their oncogenicity for rodents and the subgroup specificity of their T antigens by complement fixation assays has been extended by studies of the degree of homology among Ad genomes (GREEN et al. 1979), restriction endonuclease analysis of viral DNA, and the characterization of virion polypeptides (WADELL et al. 1980). Based upon these studies, the subgroup similarities of these viruses have been substantiated. Ad4 has been classified as the only member of subgroup E (WADELL 1979), and the enteric adenoviruses have been classified as subgroup F (WADELL et al. 1980). The nonviron early virus T antigens in Ad-induced tumors and the presence of virus-specific antibodies in animals carrying virus-free tumor cells implied the continued presence of specific regions of the Ad genome in tumor cells that were free of infectious virions. The pursuit of this implication led to much of the current understanding of the molecular structure and functional organization of the Ad genome. Many of the proteins expressed by specific Ad genes that are present in these tumor cells have been characterized, and their functions are currently being investigated (FLINT 1980a, b; PERSSON and PHILIPSON 1982).

The tumor-inducing capacity of Ad12 for Syrian hamsters and other rodents has been well documented. Sixty-one percent (314/519) of hamsters in four independent studies that used both laboratory and field strains of Ad12 developed tumors described as undifferentiated sarcomas (Table 1). Other studies have shown that Ad12 can induce tumors when inoculated into Sprague-Dawley rats, mastomys, and C3H and CBA mice (Table 1). The data used to substantiate the lack of oncogenicity of Ad2 in rodents have been less well publicized. Of the four studies of which we are aware, only two tumors have been observed in 159 hamsters inoculated with infectious Ad2 or Ad2 inactivated by exposure to ultraviolet (UV) light (Table 1). No tumors were observed in 16 Fisher or Sprague-Dawley rats or in 35 BALB or NIH Swiss mice. We are not aware of studies in which the oncogenicity of Ad2 has been tested in C3H or CBA mice. The Ad2 inoculum used to inject the hamster that developed one of the tumors listed in Table 1 (GIRARDI et al. 1964) was subsequently found to be contaminated with SV40. Cells from this tumor were not reported to have been examined for the presence of Ad2 or SV40 genetic information. The second tumor in Table 1 developed in a hamster inoculated with Ad2 inactivated with UV light (LEWIS and COOK 1979). Histopathologically, this

Table 1. Oncogenicity of Ad2 and Ad12 in rodents less than 24 h old inoculated with varying doses of virus by several routes of injection

Virus	Tumor incidence (no. with tumors/no. surviving)				References
	Hamsters	Rats	Mastomys	Mice	
Ad2	0/105	0/16	–	0/35	Gilden and Huebner (unpublished)
	0/3	–	–	–	Trentin et al. (1962)
	1/5	–	–	–	Girardi et al. (1964)
	1/41	–	–	–	Lewis and Cook (1979)
Ad12	100/183	–	–	–	Trentin et al. (1962, 1968)
	83/144	–	–	–	Yabe et al. (1962, 1963)
	89/135	–	–	–	Huebner et al. (1962, 1963a)
	42/57	–	–	–	Girakdi et al. (1964)
	–	3/10	–	–	Huebner et al. (1963b)
	–	–	2/32	7/29	Rabson et al. (1964)
	–	–	–	3/13	Yabe et al. (1964)
	–	–	–	21/24	Allison et al. (1967)
	–	–	–	5/14	Sjögren et al. (1967)

tumor was an adenocarcinoma of a skin appendage, probably of the mammary gland. Cells from this tumor did not contain Ad2 T antigens or Ad2 DNA, and hamsters carrying transplants of this tumor did not develop antibodies to Ad2 antigens. Thus this tumor would appear to be a spontaneous neoplasm. Based upon these findings, Ad2 is considered to be nononcogenic for rodents while Ad12 is considered to be oncogenic for rats and mice and highly oncogenic for hamsters.

Several explanations have been advanced for the differences in tumor-inducing capacity between Ad2 and Ad12. Since both these viruses are capable of inducing neoplastic changes (i.e., transforming) in hamster and rat cells in tissue culture, differences in their efficiencies of transformation could explain the differences in their oncogenicity. Several studies have addressed this possibility (Table 2). McAllister and MacPherson (1968) and McAllister et al. (1969) noted that nononcogenic Ad19 (subgroup D) was approximately 50 times more efficient in transforming rat embryo cells in tissue culture than was the highly oncogenic Ad12 ($10^{4.5}$ PFU/FFU for Ad19; $10^{6.2}$ PFU/FFU for Ad12). Gallimore and Paraskeva (1980) found no difference in the efficiency with which Ad2 and Ad12 transformed identical batches of rat embryo brain cells (both $10^{5.9}$ PFU/FFU); and one of us (A.M. Lewis Jr., unpublished) found that these two viruses are approximately equally efficient ($10^{7.8}$ PFU/FFU for Ad2; $10^{8.0}$ PFU/FFU for Ad12) in transforming identical batches of LSH hamster embryo cells. Thus there appear to be no inherent differences in the capacity of Ad2 and Ad12 to induce neoplastic changes in cells removed from the intact animal host that can satisfactorily explain the differences in the oncogenicity of these two viruses.

The ability of nononcogenic Ad2 and oncogenic Ad12 to transform normal rodent cells to neoplastic cells in vitro with essentially the same efficiency implies

Table 2. Evidence for the lack of correlation between tumor induction in rodents and the efficiency of transforming rodent cells in vitro by human adenoviruses

Ad serotype	Tumor-inducing capacity in vivo	Efficiency of cell transformation in vitro (log_{10} PFU/FFU)	
		Rat cells	Hamster cells
1	Nononcogenic	5.8[a]	–
2	Nononcogenic	7.6 5.9[b]	7.8[c]
19	Nononcogenic	4.5	–
12	Oncogenic	6.2 5.9	8.0

[a] Data from McAllister and MacPherson (1968) and McAllister et al. (1969)
[b] Estimated from the data of Gallimore and Paraskeva (1980)
[c] Unpublished data from A.M. Lewis, Jr

that the factors determining the tumor-inducing capacity of these viruses are related to the interactions between cells transformed by these viruses in vivo and the prospective rodent host being used to evaluate viral oncogenicity. A number of observations support this conclusion. Ad2 produced tumors when inoculated into newborn rats that were immunosuppressed with antithymocyte serum (Harwood and Gallimore 1975). Ad12 is oncogenic only when inoculated into newborn rodents or adults that have been immunosuppressed by thymectomy or treatment with antithymocyte serum or steroids (Yabe et al. 1962; Kirschstein et al. 1964; Yohn et al. 1965, 1968; Allison et al. 1967). As the virus produces tumors more efficiently in immunoimmature or immunosuppressed rodents, the degree of maturation of the immune system of the prospective host appears to play a critical role in the outcome of the oncogenic process. Several studies have considered a possible role in the tumor-producing process for those properties of cells transformed in vitro that differentiate them from normal cells. These studies have been unable to consistently associate any of these properties (i.e., cell morphology, immortality, doubling times, saturation densities, serum growth requirements, anchorage independence, proteolytic enzyme activity, and the presence of surface glycoproteins) with the differences in the tumor-inducing capacities between hamster and rat cells transformed by Ad2 and Ad12 (Gallimore et al. 1977; Gallimore and Paraskeva 1980; Cook and Lewis 1979). In spite of our inability to differentiate between cells transformed in vitro by these two viruses, syngeneic rats and hamsters readily perceive the differences that have eluded us (Table 3). All the lines of Ad12-transformed rat and hamster cells that have been tested produce tumors in syngeneic newborn animals. Furthermore, Ad12-transformed inbred hamster cells are highly tumorigenic when transplanted into fully immunocompetent adult syngeneic hamsters (Lewis and Cook 1982). In contrast, only 23% of the lines of Ad2-transformed rat cells developed by Harwood and Gallimore (1975) and Gallimore and Paraskeva (1980) produced tumors in immunoimmature newborn rats further immunosuppressed by treatment with antirat thymocyte serum. The majority (87%) of the lines of Ad2-transformed inbred hamster cells that we have tested are highly tumorigenic in immunoimmature

Table 3. Tumor-inducing capacity of Ad2- and Ad12-transformed inbred rat and hamster cells in syngeneic rats and hamsters

Transforming virus	No. cell lines oncogenic/no. cell lines tested (%)			
	ATS newborn rats[a]	Normal newborn rats	Newborn hamsters[b]	Adult hamsters
Ad2	16/70 (23)	1/70 (1)	13/15 (87)	0/15 (0)
Ad12	NT	25/25 (100)	5/5 (100)	5/5 (100)

ATS, treated with antirat thymocyte serum; NT, not tested

[a] Rat data from GALLIMORE and PARASKEVA (1980). Animals were challenged with 2×10^6 cells

[b] Hamster data from COOK and LEWIS (1979) and LEWIS and COOK (1980, 1982). Hamsters were challenged with 10^7 cells

newborn hamsters but nontumorigenic when 10^7 cells are inoculated into immunocompetent syngeneic adult hamsters (COOK and LEWIS 1979). These results strongly support the conclusion that it is the interactions between the host and Ad2- or Ad12-transformed cells that ultimately determine the outcome of the neoplastic processes which lead to tumor development.

3 Virus-Specific Immunogenicity of Ad2- and Ad12-Transformed Rodent Cells

Tumor-specific transplantation antigens (TSTAs) have been discovered on cells from tumors induced by a number of chemical carcinogens and oncogenic viruses. These antigens are detected by their ability to induce cellular immune reactions in a host immunized with tumor viruses or tumor cells, resulting in the destruction of viable antigen-containing tumor cells. The discovery of TSTAs on neoplastic cells was of major theoretical significance, as it offered an immunological approach to tumor therapy and suggested the importance of host immune mechanisms in determining the outcome of virus-induced neoplastic disease. The presence of TSTAs on the surface of tumor cells also offered an explanation for the observed differences in the tumor-inducing capacities among oncogenic viruses and the cells they transformed in vitro. This explanation suggested that the different levels of immunogenicity expressed by cells transformed in tissue cultures should evoke different degrees of immune recognition and rejection in the potential host. By this reasoning, weakly immunogenic neoplastic cells should be highly tumorigenic and highly immunogenic neoplastic cells should be weakly tumorigenic. Conversely, immunoincompetent animals should be more susceptible to tumor induction by transformed cells that express transplantation antigens than fully immunocompetent animals.

One of the first indications that the immune system of the host plays a role in tumor induction by adenoviruses was provided by KIRSCHSTEIN et al. (1964), who found that Ad12 could produce tumors only in thymectomized BALB/c or C3H mice. Shortly after this initial observation, YOHN et al. (1965)

found that thymectomy increased the incidence of tumor induction by Ad12 in male syrian hamsters, and ALLISON et al. (1967) reported that thymectomized CBA mice and CBA mice treated with antilymphocyte serum and inoculated with doses of Ad12 that were marginally tumorigenic developed a higher percentage of tumors than did untreated normal animals.

EDDY et al. (1964) and TRENTIN and BRYAN (1966) described the induction of immunity to Ad12 TSTAs in virus-immunized hamsters and mice. BERMAN (1967) demonstrated the presence of Ad12 TSTAs on cells from tumors induced by Ad12 in CBA mice and found that protection against tumor challenge was mediated by immune lymphoid cells and not by serum containing antibodies to Ad12 T antigens. SJÖGREN et al. (1967) demonstrated the presence of TSTAs on tumor cells induced in C3H mice by Ad12, and also found that immunization with Ad7 and Ad18 but not Ad5 could protect mice against a challenge with Ad12 tumor cells. ANKERST and SJÖGREN (1969, 1970) extended this observation; they found that Ad serotypes 3, 7, 12, and 14 seemed to share a common TSTA and that TSTAs on cells from tumors induced in mice and hamsters by Ad12 shared immunological specificities. These results suggested that the Ad12 genome codes for common TSTA specificities that are independent of the species of animal cell undergoing neoplastic transformation.

Thus far, the role of the viral genome in inducing TSTAs in cells transformed in vitro and in vivo by human adenoviruses has not been precisely defined. SHIROKI et al. (1979) have shown that rat cells transformed by subgenomic fragments of Ad12 (*Eco*RI C fragment map position 0–16 and *Hind*III G fragment map position 0–7.2) protected immunized rats inoculated as newborns with Ad12 against subsequent tumor development. Cells transformed by the *Acc*I/*Bpa*I H fragment (map position 0–4.5) did not protect immune rats against Ad12 tumor development. RASKA et al. (1980) found that rats carrying tumors induced by cells transformed by the same Ad12 *Eco*RI C fragment developed lymphoid cells in their spleens that were specifically cytotoxic for syngeneic but not allogeneic Ad12-transformed rat cells in vitro. The tumor-bearing animals also developed complement-dependent antibodies that were cytotoxic for Ad12-transformed cells in vitro. Ad2-transformed rat embryo cells containing only the left-hand 14% of Ad genome produced similar complement-dependent cytotoxic antibodies and cytolytic T lymphocytes in the sera and spleens of immune rats (RASKA et al. 1982). These data imply that the early region 1 (E1) of the Ad12 and Ad2 genomes encode proteins that specify TSTAs in virus-transformed cells. The E1 region of the viral genome is located between map position 1.0 and map position 11.5. Proteins encoded by this region apparently interact with cell surface histocompatibility antigens to produce a specific complex that can be recognized by cytotoxic T lymphocytes from virus-immune syngeneic animals in a manner similar to those described for the papovavirus SV40 TSTA system (TEVETHIA 1980). The E1 region of the Ad genome has been divided into two transcription units (E1A and E1B) by the presence of two distinct promoters (SEHGAL et al. 1979; WILSON et al. 1979). The E1A region extends from about map position 1.0 to map position 4.4, while the E1B region extends from about map position 4.5 to map position 11.5. Thus the data of SHIROKI et al. (1979) and RASKA et al. (1980) indicate that polypep-

tides from the E1B region of the Ad genome are responsible for the Ad-specific TSTAs present on Ad2-transformed rodent cells. PERSSON et al. (1982) have purified a 15000-dalton protein encoded by the E1B region of the Ad2 genome. Monospecific antiserum prepared with this protein immunoprecipitated a 15000-dalton protein from human and rodent cells transformed by Ad2 and Ad5. Since this protein was found to be tightly associated with cell membranes, it may be an Ad2 gene product that conveys virus-specific immunogenicity to transformed cells.

KVIST et al. (1978) found that antiserum prepared against Ad2-transformed rat A2T2C4 cells and antiserum against the rat major histocompatibility antigens immunoprecipitate the same 19000-dalton glycosylated cell surface antigen from A2T2C4 cells. Antiserum that was specific for β_2-microglobulin [a subunit of cell surface histocompatibility antigens: RASK et al. (1974); SILVER and HOOD (1974); VITETTA et al. (1975)] also precipitated the 19000-dalton polypeptide. Their data suggest that the 19000-dalton polypeptide is a virus-coded protein that forms a ternary complex with the subunits of the rat histocompatibility antigens on the surface of the A2T2C4 cells. Subsequent studies (PERSSON et al. 1980; SIGNAS et al. 1982) identified the 19000-dalton Ad glycoprotein as a product of early region 3 (E3, map position 76.0 to map position 86.0) of the Ad2 genome and showed that it binds specifically to the heavy chain of class 1 antigens of the major histocompatibility complex present on the surface of Ad2-infected human cells. These studies also demonstrated that the complex formed between this Ad glycoprotein and class 1 histocompatibility antigens are recognized by cytotoxic T lymphocytes. Thus both the E1 and E3 regions of the adenovirus genome appear to encode proteins that may be associated with the virus-specific immunogenicity of infected and transformed cells.

MCALLISTER et al. (1969) suggested that the induction of highly immunogenic transplantation antigens on rodent cells transformed in vivo and in vitro might determine the oncogenicity of the Ad serotype and the tumor-inducing capacity of the virus-transformed cell. Subsequent studies demonstrated that rat cells transformed by nononcogenic Ad2 were capable of inducing tumors only in immunosuppressed newborn rats (GALLIMORE 1972; HARWOOD and GALLIMORE 1975). Since rat cells transformed by oncogenic Ad12 produced tumors quite readily in newborn rats, these findings supported the concept that differences in the immunogenicity of rodent cells transformed by the highly oncogenic, weakly oncogenic, and nonocogenic adenoviruses could explain the differences in their tumor-inducing capacities.

The evidence obtained by successfully grafting nontumorigenic transformed cells into immunosuppressed hosts implies that some type of immunological mechanism is at work in determining the success or failure of the transformed cells in establishing themselves as a neoplasm. Such studies provide only indirect evidence that the immunogenicity of the transformed cell is the determining factor. In a direct attempt to correlate tumorigenicity and immunogenicity, AKAGI and OGAWA (1972) found no correlation between the expression of TSTAs and the "intensity of tumorigenicity" among three cell lines derived from hamster cells transformed in vitro by Ad12 and one cell line established from a tumor induced in hamsters by Ad12. In more recent studies, GALLIMORE

Table 4. Evidence for the lack of correlation between the immunogenicity and tumorigenicity of Ad2- and Ad12-transformed rat and hamster cells

Cell line	Tumor-inducing capacity							Resistance index
	Nude mice	Rats			Hamsters			
		ATS newborns	Normal newborns	Adults	ALS newborns	Normal newborns	Adults	
Ad2F4[a]	+(4.8)	+	0	–	–	–	–	>400
Ad2F17	+(7.3)	0	0	–	–	–	–	>400
Ad2F19	+(6.0)	0	0	–	–	–	–	>635
Ad250A	+(2.3)	+	+	+	–	–	–	>635
Ad2HE7[b]	+(5.5)[f]	–	–	–	+(5.5)	0(>8.5)	0(>8.5)	107
Ad2HE1	+(3.9)	–	–	–	NT	+(5.3)	0(>8.5)	1,961
Ad2HE3	+(4.9)	–	–	–	NT	+(3.6)	0(>8.5)	2,511
Ad2HE3-ATCL-1	NT	–	–	–	NT	NT	+(4.1)	1,149
Ad12HE1	+(4.1)	–	–	–	NT	+(3.4)	+(4.5)	10,000

Resistance index, no. of cells per TPD_{50} in immune animals/no. cells per TPD_{50} in nonimmune controls; ATS, treated with antirat thymocyte serum; ALS, treated with antihamster lymphocyte serum; +, tumorigenic when animals were challenged with single doses ($10^{6\cdot3}$) of transformed cells in parentheses. no. of cells per TPD at the 100% end point (top half of table) or no. of cells per TPD_{50} (bottom half of toble)]; NT, not tested

[a] Ad2-transformed rat embryo brain cells (Ad2F4, Ad2F17, Ad2F19) or Ad2-transformed rat embryo fibroblasts (Ad250A). From the data of *Gallimore* and *Paraskeva* (1980)

[b] Ad2- or Ad12-transformed inbred LSH hamster embryo cells (Ad2HE7, Ad2HE3, Ad12HE1). To establish the Ad2HE3-ATCL-1 cell line, tumors were induced in newborn hamsters with Ad2HE3 cells. These tumors were adapted to grow in adult hamsters by serial passage in newborns. After multiple passages in adult hamsters, cells from a single tumor were established in vitro

and PARASKEVA (1980) and LEWIS and COOK (1982) have attempted to correlate the expression of virus-specific immunogenicity of Ad2-transformed rat and hamster cells with their tumor-inducing capacities (Table 4). Ad2-transformed rat embryo brain cells and Ad2-transformed rat embryo fibroblasts that exhibited a variety of tumor-inducing capacities for nude mice, normal syngeneic newborn rats, and antirat-thymocyte-serum-treated syngeneic newborn rats were all highly immunogenic (resistance indices >400) in bioassays for the expression of Ad2-specific transplantation antigen. Three lines of Ad2-transformed LSH hamster embryo cells and one line of Ad12-transformed LSH hamster embryo cells that exhibited three distinct tumorigenic phenotypes – tumorigenic in nude mice and nontumorigenic in hamsters (Ad2HE7); tumorigenic in nude mice and syngeneic newborn hamsters but nontumorigenic in adult hamsters (Ad2HE1 and Ad2HE3); tumorigenic in nude mice and in syngeneic newborn hamsters and adult hamsters (Ad2HE3-ATCL-1 and Ad12HE1) – were widely divergent in their ability to convey protection to immune hamsters challenged with graded tumor-producing doses of viable transformed cells. Indeed, the least tumorigenic of the cell lines (Ad2HE7), which produced tumors only in nude mice, was the least immunogenic in repetitive assays (average resistance index of 107) in which immune animals were challenged with Ad2HE3-ATCL-1 cells. Ad12HE1 cells that produced tumors quite efficiently [$10^{4.5}$ cells/tumor-producing dose at the 50% end point (TPD_{50})] in syngeneic adult hamsters were highly immunogenic in that no tumors developed in immune hamsters challenged with more than 10000 TPD_{50} of Ad12HE3 cells. These results imply that the differences in the immunogenicity of Ad-transformed cells as reflected by their expression of TSTAs which can be detected by bioassay are not related to the differences in their tumor-inducing capacity. Furthermore, the minor differences in the immunogenicities of the Ad2HE3 cells (resistance index 2511) and the cells selected during in vivo tumor passage that become the more highly oncogenic Ad2HE3-ATCL-1 cell line (resistance index 1149) imply that the cell selection process involved in tumor induction in animals that possess a greater degree of immunocompetence does not result in the overgrowth of cells that are markedly deficient in Ad2 TSTAs.

4 Adenovirus-Transformed Cell Tumorigenic Phenotypes Defined in the Context of the Host Cellular Immune Response

By definition, bioassays evaluating virus-specific immunogenicities of adenovirus-transformed cells reveal the ability of the immunized host to respond to a repeated exposure to TSTAs. It is apparent from the above data that the elicitation of such secondary host responses does not discriminate among adenovirus-transformed rodent cells that differ in their tumor-inducing capacities. Rather, the key factor differentiating highly oncogenic Ad12-transformed cells from weakly oncogenic Ad2-transformed rodent cells appears to be the outcome of the interactions between the transformed cells and the primary host defenses mounted by the naive host. The nature of these host defenses has been examined