ADVANCES IN CANCER RESEARCH

VOLUME 24

ADVANCES IN CANCER RESEARCH

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OBITUARY

Sir Alexander Haddow
1907–1976

The Editors of this series sadly announce the loss of a distinguished colleague, Sir Alexander Haddow, whose death on January 21, 1976, ended the career of one of the world's outstanding leaders in cancer research. By a strange coincidence, another luminary in cancer research, Waro Nakahara, died on the same day. Alex Haddow was one of the founders of *Advances in Cancer Research,* and served as a coeditor with the late Jesse Greenstein from 1953 to 1958 (Volumes 1 through 5). On the death of Jesse Greenstein in 1956, one of us (S.W.) took his place, and from Volumes 6 through 11, had the privilege of collaborating as coeditor with Alex Haddow. When ill health (virtual blindness) made it necessary to terminate this role in 1968, Alex continued his association with the Advances as Consulting Editor.

His was a life uniquely devoted to cancer research. As he describes in a stirring autobiographical essay [*Cancer Research* 34, 3159–64 (1974)], his choice of a career in medicine was made when he was hardly out of the cradle. Shortly after graduating in medicine from the University of Edinburgh in 1929, he began research in chemical carcinogenesis at the same University; and continued work in this field on joining the Royal Cancer Hospital in London in 1936. At that time and place, the newly emerging field of hydrocarbon carcinogenesis was developing brilliantly under the leadership of Sir Ernest Kennaway.

Succeeding Kennaway in 1946 as Director, Alex Haddow built the newly established Chester Beatty Institute into one of the world's leading cancer centers, where epoch-making progress was recorded in chemotherapy, chemical carcinogenesis, and the biology and pathophysiology of cancer. Since 1972, when the complications of diabetes necessitated his retirement, he moved to the Institute's lodge at Pollards Woods, where with the constant aid of his wife Feo, he continued his life of study and writing.

Despite a busy research career and directorial responsibilities, Sir Alex was heavily involved in worldwide organizations devoted to the cancer problem, and his various posts in such external bodies are too many to list in these short paragraphs. He held several leadership positions

in the British Empire Cancer Campaign, was founder and President of the Oncology Section of the Royal College of Medicine, a Fellow of the Royal Society, Vice-President of the British Cancer Council, and from 1962 to 1966 was President of the International Union Against Cancer. Among many awards were foreign memberships in the Academy of Medical Science, USSR; Academy of Arts and Sciences, U.S.A.; the American Association for Cancer Research (Honorary Member); and the New York Academy of Sciences (Fellow). Other honors were received from France (Croix de Chevalier de Legion d'Honneur), Cuba, Belgium, and Czechoslovakia; and Honorary Doctorates from the Universities of Edinburgh, Perugia, and Helsinki. He was knighted in 1966.

The Editors of this serial publication mourn a warm friend, a brilliant scientist and leader of scientists, and a benefactor of humanity.

CONTENTS

THE MURINE SARCOMA VIRUS-INDUCED TUMOR: EXCEPTION OR GENERAL MODEL IN TUMOR IMMUNOLOGY?

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I. Introduction

The tumors induced by murine sarcoma virus (MSV)[1] have been one of the most extensively studied models in tumor immunology during the past 10 years. Being autochthonous tumors with a rapid development at the site of virus inoculation, followed by a spontaneous rejection and a strong resistance to further virus challenges, they appear as an attractive model for the study of the antitumor response in the natural host of a primary tumor. Furthermore, the MSV is oncogenic for various inbred strains of mice as well as for hamsters and rats, thus providing the opportunity to compare the antitumor response in different genetic backgrounds. Numerous groups have chosen this model, and we now have a considerable amount of information about the tumor-associated antigens, the antibody response, and the cell-mediated antitumor reaction. This review will try to summarize these data and the problems that now arise about the immunology of the MSV system, since these problems are of general interest in experimental and human tumor immunology. The observations reported in this system have been especially useful, notably in cellular immunology, but we still do not know whether the highly antigenic MSV tumor must be considered as a general model, or as an exception in tumor immunology.

Most of the experiments that will be reviewed, have been done with five different isolates of MSV: Harvey, or H-MSV (Harvey, 1964); Moloney, or M-MSV (Moloney, 1966); Kirsten, or K-MSV (Kirsten and Mayer, 1967); Finkel, or FBJ-MSV (Finkel *et al.*, 1966) and Gazdar or G_z-MSV (Gazdar *et al.*, 1972a). However, it must be emphasized that the descriptions of the antitumor response, notably when cell-mediated, concern mainly the M-MSV isolate, probably because it is characterized

[1] Abbreviations used in this review: MSV: murine sarcoma virus; MSV prefixed with H, K, M, Gz or FBJ: MSV pseudotypes isolated, respectively, by Harvey, Kirsten, Moloney, Gazdar, or Finkel; MuMAV: murine myeloma-associated virus; MuLV: murine leukemia virus; G, Gi, F, M, and R, respectively, Gross, Graffi, Friend, Moloney, and Rauscher strain of MuLV; WMV: woolly monkey virus; TATA: tumor-associated transplantation antigen; SCSA: sarcoma-specific antigen; GCSA: Gross specific cell surface antigen; VCSA: viral cell surface antigen; VEA: viral envelope antigen; MEV-SA: murine endogenous viral surface antigen; gs: group-specific antigen; NP (cells): nonproducer (cells); GVH: graft-versus-host reaction; CTL: cytolytic T lymphocytes; CMI: cell-mediated immunity; IEM: immunoelectron microscopy; IF: immunofluorescence; CIT: colony-inhibition test; MA: microcytotoxicity assay; MLTR: mixed lymphocyte tumor cells reaction; CRT: chromium release test; S. CRT: secondary CRT; MMI: macrophage migration inhibition test; PA: proline assay; ATS: antithymocyte serum; ADCC: antibody-dependent cell-mediated cytotoxicity.

by (1) usual induction of sarcomas at the site of virus inoculation without other macroscopically detectable pathology, (2) regular spontaneous rejections, which are less frequent with the other isolates (Harvey and East, 1971).

The pathology and virology of MSV will not be considered in this review. They have been extensively studied in the Harvey and East review (1971). In addition, a great number of subsequent reports have been published, which cannot be reviewed here. However, it is necessary to know the main characteristics of MSV, a type C RNA virus with defective replication and transforming activities *in vivo* and *in vitro*, to comprehend the immunologic aspects of the MSV system. The constant association of a helper virus in MSV producer cells must be especially emphasized. Also, no comparison has been attempted with the immunology of other tumors, unless necessary.

As far as possible, each of the four main sections of the review has been treated as a unit in its own right. The sections concern, respectively, *in vivo* tumor protection (Section II), antivirus immune response (Section III), antitumor cell antibody response (Section IV), and cell-mediated antitumor immunity (Section V). The complexity of the antigens of the MSV system is remarkable. Therefore, we felt that to ensure a better understanding, the antigens involved in each of these four reactions must be studied separately. In addition, Table I summarizes the main antigens existing in the MSV tumor.

II. *In Vivo* Studies of the Immunological Rejection of MSV-Induced Sarcomas

A. Suggestions of an Immune Reaction from the Natural History of the Tumor

The evolution of the tumors induced in mice by subcutaneous or intramuscular MSV inoculation is well known (Harvey and East, 1971). The neoplasms arise and progress rapidly at the site of inoculation, and they are frequently extensive enough to weigh up to 10% of the total body weight when newborns have been inoculated. One of the most remarkable points is that these tumors will follow a different evolution in very young and in adult animals. Whatever the inbred line, practically 100% of the adults will finally reject the local M-MSV tumor. The pathology of H-MSV and K-MSV is more complex since most of the treated animals develop at the same time a local tumor and a spleen erythroblastosis. Changes similar to those of Friend disease with erythroblast proliferation are usual after H-MSV infection, so that the mice

TABLE I
MAIN ANTIGENS OF MSV TUMOR CELLS

Nature[b]	Antigens	Situation in the virion	Cell-localization	Molecular support	Directing genome	References	Observations
VEA	Type specific *VEA*	Surface projections	(1) Budding particles	gp69/71	Helper virus	Eckner-Steeves (1972)	Another sub-gs VEA *Mulv-VEA* was described by Aoki 1974
	gs-VEA	Surface projections	(2) Can be VCSA	gp69/71	Helper virus	Gomard *et al.* (1973) Aoki (1974)	
VCSA	*FMR* or FMRGi	Internal	Cell surface outside viral particles	? (p15?)	Helper virus	Old *et al.* (1964)	FMRGi and GCSA (a) are subgroup specific
	GCSA (a)	?	Cell surface outside viral particles	? (p15?)	Helper virus	Old *et al.* (1965)	
	GCSA (b)	?	"	?	Helper virus	Geering *et al.* (1966, 1968)	GCSA (b) gs1, and gs3 are group specific GCSA (b) could be identical to gs1
	gs1	Core shell	"	p30	Helper virus	Yoshiki *et al.* (1973, 1974)	
	gs3	Core shell	"	p30	Helper virus	Ferrer (1973) Aoki *et al* (1973)	
SCSA	SCSA	?	Cell surface in nonproducer cells and cell surface outside virus particles in producer cells	?	MSV	Aoki *et al.* (1974a)	
	SCSA (d)						
	SCSA (b) and (c)						
Embryonic antigens	Embryonic specificity	Absent ?	Cell surface	Cellular components	Host cell	Salinas and Hanna (1974)	Could be multiple
Endogenous virus antigens	MEV-SA1	?	Cell surface	?	Endogenous type C virus	Herberman *et al.* (1974)	

[a] For general reviews, see Levy (1974) and Bauer (1974).
[b] VEA, viral envelope antigens; VCSA, viral cell-surface antigens; SCSA, sarcoma cell-surface antigens.

generally are killed by the spleen lesions without having had the time for regression of the local tumor. On the contrary, when M-MSV is used, there is no spleen erythroblastosis and the evolution of the local tumor can be studied independently. After 2–4 weeks, on the average, all the animals are tumor-free and spontaneous recurrences occur only in a very small percentage. On the other hand, 100% of the newborn infected recipients die with a huge local tumor, sometimes with metastatic proliferation (Harvey and East, 1971). It is interesting to observe that sarcomas appear in adults as well as in newborns of the same inbred line when high virus doses are inoculated, the discrepancy between the two groups being detectable only at the stage of the tumor rejection. This suggests that the cells are sensitive to the oncogenic potency of MSV in adults as well as in newborns, but only adults are able to mount an antitumor reaction. From the beginning, it was supposed that this reaction could be immunologic, and that the newborns do not reject MSV tumors owing to their well-known immunologic immaturity.

The ontogeny of the antitumor response has been studied by different groups (Fefer, 1969; McCoy *et al.*, 1972a). The anti-M-MSV response becomes detectable *in vivo* in BALB/c around the age of 3 weeks. A 50–70% rejection is observed at 4 weeks, and the maximum a little later on. However, the rejection ability may still not be total at the age of 8 weeks (Fefer, 1969). In CBA/wh the resistance to M-MSV is detectable at 2 weeks and complete at 5 weeks. A similar, or slightly more rapid, development of the antitumor response has been found in C3Hf/Gs inoculated with K-MSV, with complete protection in mice 4–5 weeks old (McCoy *et al.*, 1972a). In our experiments (unpublished results), C57BL/6 are especially remarkable by a very rapid appearance of the ability to reject the M-MSV tumor, all being already rejected in 2-week-old recipients.

The level of sensitivity is different among the inbred strains of mice. For instance, C57BL/6, C57BL/10, B10-Br, DBA/2, CBA, Swiss NIH, and BALB/c are sensitive, whereas AKR and their F_1 hybrids with CBA, NIH, or DBA/2 are relatively resistant to the M-MSV (Chieco-Bianchi *et al.*, 1974; Colombatti *et al.*, 1975a,b). Even among sensitive lines some discrepancies can be found: it is well known, for instance, that C57BL/6 are less sensitive than BALB/c to low virus doses, and that they reject the tumor more rapidly. These variations could be due to unequal levels of antitumor immune response, but no precise arguments have been yet given to support this hypothesis.

The study of the tumor histology reinforces the idea that the tumor rejection could be an immunological phenomenon. Two different types of lesions can be found in the tumors: a clearly neoplastic proliferation

and an inflammatory granulomatous reaction. The neoplastic proliferation is composed of mesenchymal cells that can be fibrosarcomatous, or myoblast cells, or other mesenchymal, sometimes undifferentiated cells. In addition, hemangiosarcomas appear also relatively frequently (see notably Chesterman *et al.*, 1966; Perk and Moloney, 1966; Perk *et al.*, 1967; Stanton *et al.*, 1968; Thomas *et al.*, 1973; and for a review, Harvey and East, 1971). This problem will not be discussed here, but it can be mentioned that no differences in the antitumor response have been demonstrated according to the cell type of the neoplastic proliferation.

The inflammatory reaction consists of polymorphonuclear cells, occasionally mast cells, and eosinophiles and a dominant infiltration of mononuclear cells, which are lymphocytes and possibly histiocytes. During tumor evolution in adults, this inflammatory exudate becomes more and more important, whereas the number of tumor cells decreases. Finally, the tumor cells completely disappear. On the contrary, the study of tumors induced in newborns does not reveal any mononuclear cell infiltration, but only the proliferation of tumor cells, which progress continuously until death (Perk and Moloney, 1966; Fefer *et al.*, 1968a). The observation of tumor cell grafts confirms the correlation between mononuclear infiltration and the ability to reject the tumor (Russel and Cochrane, 1974), and the same conclusions are drawn from the study of G_2-MSV tumors (Gazdar *et al.*, 1973). Therefore, one can suppose that this infiltration represents an antitumor reaction that will provoke the tumor cell destruction. This hypothesis is strengthened by the observation that lesions with the usual morphologic characteristics of neoplasms, that is to say, with large areas almost exclusively composed of cells of the same type, with mitotic foci and no apparent organization, are rare in adult infected mice, but occur more frequently in thymectomized or irradiated animals (Stanton *et al.*, 1968). This kind of proliferation, with a clear neoplastic appearance, is especially frequent in tumors that develop several weeks after virus inoculation. Similarly, in addition to typical pleomorphic tumors, other neoplasms, composed of monomorphic cells with nodular or diffuse growth, reminiscent of clonal aggregates, can be observed in the resistant adult AKR inoculated with M-MSV (Chieco-Bianchi *et al.*, 1974). In these mice, the tumors grow slowly, but they ultimately kill the host in most cases; they are due to the spontaneous formation of a poorly immunogenic Gross (G) pseudotype (see Section III,A,1). Similarly, the naturally occurring G pseudotype of the FBJ-MSV isolated from a spontaneous osteosarcoma of CF1 mice (Finkel *et al.*, 1966), induces progressively growing tumors with purely neoplastic morphological characteristics and very few granulomatous lesions or mononuclear cell infiltrations (Price *et al.*, 1972). *Therefore, when one*

considers the value of MSV tumors as an in vivo model in tumor immunology, one must remember that two different kinds of such tumors exist:

1. The sarcomas, detectable very early after the virus inoculation, usually in the first 2 weeks, are virus-producing and strongly antigenic. In most cases, adults are able to reject these tumors, which are associated with an inflammatory reaction. It is not certain whether or not the tumor cells are really autonomous; a constant production of virus with recruitment of newly infected transformed cells could be necessary to ensure tumor development, as suggested notably by the difficulty in establishing permanent transformed cell lines by *in vitro* infection of primary mouse embryo fibroblasts or in maintaining primary *in vivo* MSV-induced tumors in a permament *in vitro* culture (Simons, 1970; Simons and McCully, 1970). In some way, these early sarcomas are perhaps equivalent to the "Early Foci," dependence of virus production in the *in vitro* MSV-induced transformation (Aaronson *et al.*, 1970). It is probable that really autonomous tumor cell clones would also appear inside these early sarcomas. However, in most cases, such clones would be superinfected by the viruses produced by the surrounding cells, and therefore they would be destroyed by the antitumor response, which appears to be mainly directed against viruses and/or virus products of the host cell surface (see following sections of this review).

2. By contrast, late sarcomas, which appear after several weeks could be the *in vivo* equivalent of the *in vitro* virus-production-independent "late foci" of transformed cells (Aaronson *et al.*, 1970). Such sarcoma cell clones would be selected mainly in two situations: if they are non-virus producers or if they produce a poorly immunogenic virus. In both cases, it would not be surprising if the mononuclear cell infiltration were absent or remained very weak, which could explain the slow but continuous proliferation.

The rapidly growing sarcomas provide very convenient systems for study of the rejection of tumor cells *in vivo,* but the slowly growing sarcomas are probably much more relevant for the natural situation.

B. *In Vivo* Demonstration of a Potent Antitumor Response in MSV-Tumor-Bearing Mice

1. *Development of a Specific anti-MSV Tumor Resistance in Regressor Mice*

Regressor mice are strongly immunized against a booster MSV injection (Fefer *et al.*, 1968a) or against the graft of live sarcoma cells (Fefer *et al.*, 1967a; Burstein, 1970). The same is true in regressor rats (Jones