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Edited by

GEORGE KLEIN

SIDNEY WEINHOUSE

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I. Introduction

The biology of cancer has been an area of intensive investigation for several years. Cancer remains one of the most challenging fields in the twentieth century and the most enigmatic. Research in cancer has attracted scientists from numerous disciplines and has provided the impetus for investigations in molecular biology, biochemistry, and immunology.

In the field of immunology, interest in cancer research was awakened by the introduction of the concept of "immunological surveillance" by Burnet (1971). This concept suggests that, normally, tumor cells arise spontaneously but are eliminated by the host immune response. However, tumors would

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develop and grow when there is a failure of the host immune response. The presence of host immune response would suggest that tumor cells must express neoantigens which are recognized by the host as foreign.

Thus, tumor immunology has developed into an intensive area of research investigations in an attempt to provide evidence for the Immune Surveillance Theory. In addition, several studies have been done to implement these concepts in the diagnosis and treatment of cancer.

Convincing evidence of immune surveillance has been provided in areas of neoantigens, host immune response, and regulation. However, the evidence was primarily derived from experimental studies in animals using virally induced tumors or long-term transplantable or chemically induced tumors. Little information was available using primary tumors of spontaneous origin. Therefore, one important challenge in tumor immunology is to investigate primary spontaneous tumors and to determine whether they behave similarly to or differently from experimental tumors. Such studies would provide the means to investigate several fundamental questions of clinical significance in the field of cancer immunology.

The spontaneously arising reticulum cell sarcoma (RCS) of SJL/J mice is a good experimental tumor system for investigation for the following reasons: (1) the tumor is spontaneous and resembles Hodgkin's lymphoma and is thus of clinical importance; (2) the majority of mice develop RCS by the age of 8–12 months, although a small percentage fails to develop the tumor, which suggests that possibly, with some of the mice, the host immune system may play a role in tumor arrest; (3) the role of an antitumor immune response was corroborated in studies showing resistance to transplantable RCS in mice immune to the tumor (Murphy, 1969); these studies suggested that RCS tumor cells may express neoantigens; (4) the exact histological nature of the tumor is not well defined and has been classified as a type B reticulum cell sarcoma (Dunn, 1954). The heterogeneity of the tumor with an unknown cell origin resembles several ill-defined spontaneous tumors in man. Since the RCS tumor system delineated above offers several unique features, several studies have provided experimental evidence for new concepts in the biology of SJL/J RCS. For instance, the strong proliferative response induced by the tumor in the syngeneic host, the expression of inappropriate alien major histocompatibility complex (MHC) antigens by the tumor, and the strong dependency of host cells for tumor growth are only a few examples that have emerged with this tumor system. This article will attempt to summarize present findings (published and unpublished) and establish a possible model(s) of host–tumor interrelationship which takes into account the available information at hand.

II. Histopathology and Characteristics of SJL/J Reticulum Cell Sarcomas

A. Pathogenesis

Murphy (1963) found that a new inbred strain, SJL/J, had a high incidence (up to 91%) of reticulum cell neoplasms at a mean age of 13.3 months. The basic histological pattern of the neoplasms was that of type B as described by Dunn (1954). Haran-Ghera *et al.* (1967) have reported that the incidence of RCS did not vary greatly between males and females (71 – 78%, respectively), with an average latent period of 348 – 380 days. Lesions were restricted to the mesenteric and cervical lymph nodes, Peyer's patches, and white pulp of spleens. More advanced lesions involved other lymph nodes, the liver, kidney, and ovaries. Twenty-five percent of the animals had marked thymus enlargement due to spontaneous reticulum cell invasion. Thymectomy, splenectomy, and castration had no effect on the incidence of tumor or latent period.

Siegler and Rich (1968) have studied the pathogenesis of RCS in SJL/J mice. They found that the neoplasm arises in the mesenteric lymph node and in Peyer's patches. The neoplastic reticulum cells grow in clusters that resemble the normal germinal centers of lymph nodes. Following a period of cell proliferation, most of the neoplastic cells die and are replaced by fibrous scar tissue. They observed that a progression of tissue changes takes place at sites previously occupied by neoplastic cells. The pleiomorphic histologic picture characteristic of RCS seems to result from the juxtaposition of clusters of proliferating cells and giant cells, and fibrous tissue products.

B. Origin

The origin of the RCS tumor has not been convincingly delineated, although several suggestions have been made. Based on the enlargement of the mesenteric lymph node at times, when all other tissues of the body were normal, Siegler and Rich (1968) suggested that the tumor is first observed in this location. It was not possible to determine whether tumors at other sites arose from metastasis by *in situ* neoplastic change. In addition, Siegler and Rich have reported that the cells that comprise the neoplasm mimic in appearance and growth characteristics the reticulum cells of the normal germinal follicles. Like the reticulum cells of normal germinal follicle cells, the tumor cells are associated with macrophages that contain cell fragments and, in some instances, nuclear fragments. Using histological criteria alone, it is relevant to speak only of neoplastic tissue rather than neoplastic cells,

because cell for cell, there is as much variation between normal reticulum cells as between normal versus neoplastic cells. This close association is likely due to the fact that both cells are premature, relatively undifferentiated cells of the mesenchyme and have the most rudimentary developmental structure. The histologic appearance of the tumors suggests that RCS may be a tumor of the "germinal follicle cells" themselves. This is of interest because these cells are regarded by many as a site of antibody production, which means that this tumor could be a neoplasm of the antibody-producing apparatus itself. The findings of altered serum globulins early in this neoplasm by Wanebo *et al.* (1966) are of interest in this regard.

Although the cellular identification of RCS in culture was not possible, the identification of the RCS tumor *in vivo* is much more complex. Several observations lend supporting evidence to a presumptive B-cell origin for these tumors. The tumor is first detected in germinal centers, including lymph nodes and Peyer's patches (Murphy, 1969). These misshapen large cells might be histiocytes or they might be early stages of B cells (Lukes and Collins, 1975). Homing experiments with labeled SJL/J tumor cells by Carswell *et al.* (1976) showed typical B migration to lymph node follicles and splenic white pulp. However, these cells might have been normal contaminants present in the mixed tumor cell populations used for inoculation. In addition, studies by Katz *et al.* (1980b) have shown that neonatal mice treated with anti-IgM serum did not develop spontaneous RCS. These results suggested that RCS may be of B-cell origin. Alternatively, RCS may require a cell of B-cell origin for its development. Last, the ability of these tumors to stimulate a strong proliferative response by syngeneic T lymphocytes (Lerman *et al.*, 1974), and the presumptive presence of Ia antigens on SJL/J RCS (Wilbur and Bonavida, 1981) also imply that it may be of B-cell origin but does not exclude a macrophage-like cell.

Recently, Ford *et al.* (1981) showed that when tumor-bearing lymph nodes were placed in cell culture, colonies of adherent cells grew slowly to confluence and exhibited morphologic and functional properties of macrophages. The "tumor cells" were also grown in soft agar where clusters and colonies of large binucleated cells predominated. These were nonspecific esterase positive, suggesting a macrophage origin. Although these studies were interesting in delineating the origin of the SJL/J neoplasms, the critical experiments were not done (i.e., to demonstrate that the colonies obtained *in vitro* are tumorigenic and can induce a disease *in vivo*). Two reports have also suggested that RCS may be derived from clones of natural killer (NK) cells (Chang and Log, 1980; Fitzgerald and Ponzio, 1979, 1981). Of interest, only established transplantable tumors showed cytotoxic activity against NK-sensitive targets, whereas primary tumors showed no activity. Since NK cells and the tumor cells are both null-like cells, but the RCS tumor is Ia^+ whereas

NK cells are Ia$^-$, the difficulty in identifying the tumor cell raises the question as to whether the NK activity is a contaminant cell of host cell origin (Chang, 1980). In addition, Ponzio *et al.* (1980) have suggested that SJL/J RCS can produce interferon known to enhance natural killer activity. Our studies with different transplantable lines and with *in vitro* tumor lines showed no significant NK activity accounted for by tumor size (unpublished). Therefore, the exact cellular origin of RCS remains unanswered and awaits the development of specific tumor cell markers for identification and characterization.

C. Etiology

Studies to demonstrate a viral etiology for RCS have not been successful (Chang *et al.*, 1974, 1975). Yumoto and Dmochowski (1967) reported virus of the murine leukemia virus (MuLV) type in cases of primary SJL/J disease, but virus of this morphological and antigenic type is widespread and ubiquitous in mice, so there is no firm ground to implicate it in SJL/J disease. Haran-Ghera *et al.* (1967) reported transmission by filtrates inoculated into subcapsular renal implants of thymus, which suggests a viral etiology. In their experience, RCS lines have not shown MuLV particles and they lack MuLV (Gross) viral antigens (Wanebo *et al.*, 1966). Although intracisternal A particles occur in these lines, their significance is obscure. Thus, the viral etiology of RCS has not been established.

D. Maintenance of Tumor Lines *in Vivo* and *in Vitro*

a. In Vivo. Reticulum cell sarcomas show unusual transplantation behavior. There is limited initial transplantability and continual instability of transplant lines (Murphy, 1969; Haran-Ghera *et al.*, 1967). With passages, the latent period of tumor growth decreases and transplantable lines can be obtained with a relatively short latent period. In general, the tumor during serial passages retains the histological morphology of the original RCS.

b. In Vitro. Although several spontaneous RCS lines have been transplanted *in vivo* and maintained transplantability, the fact that the tumors are pleiomorphic raises logistic questions for their analyses. In addition, the concomitant presence of normal blast cells confounds characterization of the tumor cells. Although a homogeneous populationof RCS cells would aid in the study of this neoplasm, tissue culture lines of spontaneous SJL/J neoplasms have been difficult to establish. However, three transplantable *in vivo* lines derived by us from spontaneous RCS were passaged 2 to 25 times and then established in culture (Owens and Bonavida, 1977).

The initial growth of the tumor in culture was absolutely dependent upon glutathione. Two of the lines, RCS-LA6 and RCS-LA8, after 25 passages *in*

TABLE I
CHARACTERISTICS OF SJL/J RCS ESTABLISHED IN CULTURES[a]

Characteristics	Reticulum cell sarcoma tumor line		
	LA-1	LA-6	LA-8
1. Surface markers			
H-2[s]	+	+	+
Thy-1.2	−	−	−
Fc receptor	−	+	+
Surface Ig	−	−	−
Complement receptor	−	−	−
Cytoplasmic Ig	−	−	−
2. Histochemistry			
Wright's stain	Blast	Blast	Blast
β-Glucoronidase	+	−	−
Methyl-green pyronin	+	+	+
Lipase	+	+/−	−
PAS	−	+	+
Peroxidase	−	−	−
3. Phagocytosis	−	−	−
4. Transplantability	−	+	+

[a] Owens and Bonavida (1977).

vitro, lost their sulfhydryl dependence although RCS-LA1 retained the requirement for over 9 months. All three cultures were of blast-like morphology by light and electron microscopy and produced the type B neoplasms (Dunn Classification, 1954) when injected into young SJL/J mice. The identity of these cultured tumors was investigated by surface markers, histochemical staining, and cytotoxic function. The results are summarized in Table I. The tumors could not be classified as typical T, B, or monocytes, but rather null-like cells. Electron micrographs suggested that the tumors are blast cells with euchromatic nuclei, aggregated polyribosomes, and high nuclear–cytoplasmic ratios. It was concluded from the blast morphology, swollen mitochondria, and detectable endoplasmic reticulums that the cells were metabolically very active which is expected from cells growing in culture. The *in vivo* administration of the cultured RCS led to classical type B neoplasia of heterogeneity similar to spontaneous RCS. These observations are interesting since they raise the question as to whether there is a single or multiple malignant cell type(s) in this neoplasm. Conceivably, the observed *in vivo* heterogeneity may be due to (a) host response to the tumor, (b) pluripotentiality of RCS tumor cells, and (c) nonclonality of lines. Because of the lack of a suitable marker specific for RCS, these hypotheses have not been

tested experimentally. In addition, the *in vivo* transplantability of the RCS lines was lost and new lines must be generated for further studies.

E. Relationship between SJL/J RCS and Hodgkin's Disease in Man

The SJL/J tumor system closely resembles Hodgkin's disease in man. This includes the classical Reed–Steinberg type cells. Eosinophils are not common in type B neoplasms of old mice of other strains, but they can be very prominent in some tumors of SJL/J mice. Extensive fibrosis does occur in the mouse in spontaneous type B tumors, and particularly in regressing transplants (Murphy, 1969). Siegler and Rich (1968) have described several common features between RCS and Hodgkin's. Both are neoplasms of lymph tissues with a complex histologic pattern of growth and pleiomorphic cell characters. Following tumor tissue necrosis, plasma cell and giant cell granulomatosis ensues. The bulky tumor size represents only a few proliferating tumor cells. Rarely, the neoplastic cells disseminate to the bloodstream. Significant perturbation of several immunological responses is frequently seen in both SJL/J RCS and Hodgkin's disease.

Even though such similarities exist, the complexity of Hodgkin's tumors and SJL/J RCS tumors may reveal differences between these two neoplasms. Thus, selection of SJL/S RCS tumor as a model for Hodgkin's disease must be considered cautiously.

III. Development of RCS *in Vivo* and Host Cell Infiltration

The kinetics *in vivo* of tumor growth and the characteristics of the cells infiltrating the tumor were analyzed by estimating the relative frequency of various cell types in the neoplastic organs (Rand and Bonavida, unpublished).

The demonstration that RCS tumors are null-like cells lacking detectable T and B surface markers with the concurrent demonstration of antigens that immunologically cross-react with alloantigens (see Section VII) allowed the identification of tumor cells admixed with other cells. The *in vivo* kinetics of development of RCS LA-6 with other host cells was examined using density gradient centrifugation to separate cells on the basis of their specific gravities (Table II). Clearly, following transplantation, the number of null tumor cells increases steadily and by day 10 more than 90% of the cells are tumor cells. In contrast, the number of B cells declines with time. The percentage of T cells, however, remains constant initially, but declines by days 7 and 10. The size of the tumor-bearing spleens increases more than 10 times, and therefore the T cells must have proliferated significantly. The majority of T cells are blasts. Such cells were not observed in normal spleen cells. It may be that

TABLE II
KINETICS OF DEVELOPMENT OF RCS-LA6 AND CHARACTERIZATION OF HOST CELLS IN TUMOR-BEARING SPLEENS[a]

Day	Cell[b]	Unfractionated	Percentage cells in fraction of specific gravity[c] 1.070–1.080	1.065–1.070	<1.065
3	B	56	48	68	45
	T	42	42	31	44
	Null	2	14	1	11
5	B	30	34	18	16
	T	43	46	46	38
	Null	27	20	36	46
7	B	13	11	9	9
	T	29	26	30	19
	Null	58	63	61	68
10	B	25	24	23	8
	T	0	2	8	3
	Null	75	74	69	89

[a] SJL/J mice 6–8 weeks old were injected intraperitoneally with 10^6 LA6 cells/mouse. Mice were sacrificed at various time intervals and then spleens were prepared for separation on density gradients.

[b] Indirect immunofluorescence was used to characterize the cell tumor phenotype. B cells were identified by anti-Ig serum, T cells with anti-Thy-1.2, and null cells by C57BL/6 anti-BALB/c alloantiserum (anti-H-2^d antibodies cross-reactive with RCS).

[c] Low- and high-density stock solutions were prepared using Ficoll 400 (Pharmacia), sodium metrozoate, and Hanks' balanced salt solution with some modification to account for increased osmolarity of mouse cells. Gradients of constant pH were used. Three fractions were selected for studies of different specific gravities.

these blast T cells represent an amplified immune response against the neoplasm, consistent with findings of the strong proliferative T-cell response induced by RCS cells *in vitro*.

IV. Immune Competence of Normal and Tumor-Bearing SJL/J Mice

In many stems, tumor-bearing mice have been shown to have a generalized defect in immune functions and such immunodeficiencies appear to accentuate tumor growth in the compromised host. The immune competence of normal and tumor-bearing SJL/J mice was investigated in an effort to delineate whether RCS tumor growth is the result of a generalized immune deficiency (Table III).

The result of such studies indicated that the T-cell-mediated and the B-cell responses are not impaired in normal or tumor-bearing mice of different ages. In addition, there seems to exist an age-associated decrease in immunological regulation as indicated by an enhanced immune response in old mice,

TABLE III
IMMUNE COMPETENCE OF NORMAL AND TUMOR-BEARING MICE[a]

Immune responses	Normal			Tumor bearing	
	2–5 months	6–10 months	11–16 months	6–10 months	11–16 months
T-cell response					
Syngeneic MLTI	+	+	+	+	+
Syngeneic cytotoxic T cells to RCS	−	−	−	−	−
Allogeneic CMC (T)	+	+	+	+	+
T helper (for IgG antibody)	+	+	+	+	+
PHA, Con A	+	+	+	+	+
B-cell response					
Cytotoxic antibody to alloantigens (IgG and IgM)	+	+	+	+	+
Tolerance induction to HGG	+(<4)	−	−	−	−
Natural Ab to poly(I-C)	−(<4)	+	+	+	+

[a] From Owens and Bonavida (1976) and Owens (1977).

the inability of these mice to be rendered immunologically tolerant to soluble antigens (human γ-globulin) after 2 months of age, and their unusual reactivity to endogenous dsRNA (Owens and Bonavida, 1976). While these studies showed that SJL/J mice have a good response to alloantigens, other investigators have reported that SJL/J mice have deficiencies in graft-versus-host reactions, delayed type hypersensitivity, and skin allograft rejection (Haran-Ghera *et al.*, 1973). Therefore, it seems that there might be a dichotomy in the ability of SJL/J tumor-bearing mice to mount certain immune responses but not others. The inability to induce tolerance to human γ-globulin (HGG) in SJL/J mice is an interesting phenomenon previously seen with the NZB/W strain but not others. The results are consistent with reports by Fujiwara and Cinader (1974) using normal SJL/J mice less than 4 months old. The mechanism of this phenomenon is not clear, though either the loss of regulatory cells or amplification of the helper T cells may be involved. One might predict a high incidence of autoimmunity to be consistent with loss of regulation, but SJL/J mice do not show any classical symptoms of autoimmune disorders.

V. Transplantation Resistance of RCS *in Vivo*

The SJL/J RCS tumors have shown unusual transplantation behavior. Murphy (1969) has observed that RCS tumors have limited initial transplantability and there was continued instability of the transplanted lines. He

suggested that this may be explained by a host reaction to a tumor-specific antigen. A host reaction has been demonstrated histologically. In addition, evidence for the existence of tumor-specific antigen has been demonstrated by the reinoculation of the tumors in animals in which the tumors had previously failed to grow. Carswell *et al.* (1970) have also examined the presence of tumor-specific antigens in RCS. Irradiated cells of each of five established RCS lines protected syngeneic recipients against transplants of the same RCS. Resistance was limited to the RCS used for immunization and was not elicited by inoculation of similarly irradiated normal tissues, SJL/J leukemias, or a different RCS. Transplantation resistance was adoptively transferred to syngeneic recipients by viable lymph nodes, spleen, or peritoneal cells from resistant immunized donors. The mechanism underlying the tumor-specific resistance has not been delineated.

VI. The Host Immune Response against SJL/J RCS Tumors

Table III summarizes the findings discussed in more detail below.

A. The Syngeneic Mixed Lymphocyte Tumor Interaction as Measured by Proliferation

1. In Vitro *Studies*

SJL/J RCS from different sources, spontaneously occurring, transplantable *in vivo,* and cultured *in vitro* have been shown to stimulate the proliferation of syngeneic lymphocytes in mixed lymphocyte tumor interaction (MLTI). The MLTI profile appears to be different from the primary allogeneic mixed lymphocyte reaction (MLR) (Owens, 1977). The kinetics of MLTI are similar to allogeneic reactions only in that peak responses occur at 96 hr of culture. However, the magnitude of [^{3}H]thymidine incorporation is generally greater than that found in MLR at all incubation times up to 120 hr (Fig. 1). Furthermore, the syngeneic MLTI requires one-tenth the number of stimulators as compared to the MLR. These results then suggest that *in vivo* priming might have taken place in the normal SJL/J mice and the results observed would represent a memory response. However, a decrease in the number of stimulating cells required for secondary proliferation has not been observed in other allogeneic systems although an accelerated response is seen.

As indicated above, the high proliferation seen with normal SJL/J lymphocytes may represent an anamnestic response. This was tested by priming the mice with tumors before rechallenge *in vitro.* These studies showed that the syngeneic proliferation follows kinetics similar to a secondary allogeneic