



AKADÉMIAI KIADÓ

Self-defense of human sarcoma cells against cytolytic lymphoid cells of their host

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ABSTRACT

Metastatic human sarcomas temporarily respond to radio-chemotherapy relapse and remain highly resistant to further combination chemotherapy as to a curative effect, including checkpoint control.

KEYWORDS

Burnet's surveillance, natural hybridoma, myxofibrosarcoma, oncolytic virus, apoptosis, NK cells

REVIEW PAPER

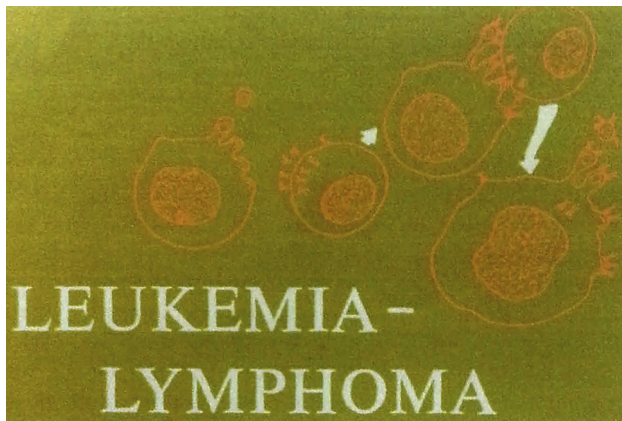


ALL IMMUNE FACULTIES RESIDE IN LYMPHOID CELLS

From the mid-1950s on, tissue cultures of human tumor cells (frequently sarcomas) exposed to various classes of lymphocytes of their hosts were the subject matter of extensive studies at the Section of Clinical Tumor Virology and Immunology, Department of Medicine and Hematology, The University of Texas MD Anderson Hospital and Tumor Institute, Houston TX. In vitro and in vivo “killer lymphocytes” including immune T cells and large granular lymphoid cells first referred to as “Burnet's immune surveillance cells” (later: natural killer cells) could slightly-to-significantly prolong remissions but failed to cure the metastatic disease. Our laboratory closed ranks equal to several others, yet received much reduced support from Rauscher's NCI. Cancer chemotherapy was declared to be eventually curative of the disease, thus it was favored by Emil Frei and Emil Freireich of the National Cancer Institute. Randolph Lee Clark's MD Anderson Hospital was enabled to house The Department of Developmental Therapeutics transferred from the Natural Cancer Institute. Yet, it was Jordan Gutterman's et al. clinical trials with human interferon (hIF) that set up the first glorification for cancer immunotherapy awarded to the Department of Developmental Therapeutics. Within the Department of Medicine, the Section of Clinical Tumor Virology and Immunology was operational without external funding, thus without access to investigational hIF (murine mIF was available). Yet it was the site where the first natural “hybridoma” and the large granular human lymphocytes first called “Burnet's immune surveillance cells” were discovered in the 1960s (*vide infra*). These projects were institutionally registered by Joseph Sinkovics (but were not externally funded). The artificial hybridomas become later the worldwide source of therapeutic monoclonal antibodies of Kohler and Milstein (without any citation to J Sinkovics et al. Lancet 1:139–140:1970; Bull Mol Med 26:61–80;2005). The large granular lymphocytes have become later (1975) the “natural killer cells” (R Kiessling and Eva Klein et al.), but without any citation ever to the original photographs of our Fig. 1 of 1969–1970 (*vide infra*). These events are historically recorded here (Int J Virol AIDS 7:072 volume 7 issue 3, 2020; Ann Clin Lab Sci vol 49, no 6, 691–698; 2019).

In our academic past, stood the “oncolytic viruses”: would it be possible to use non-pathogenic viruses for therapeutic oncolysis? Our first attempt in the USA was highly promising as recalled later (Internat J Virology AIDS 2019, 6:049 volume 6 issue 1). While

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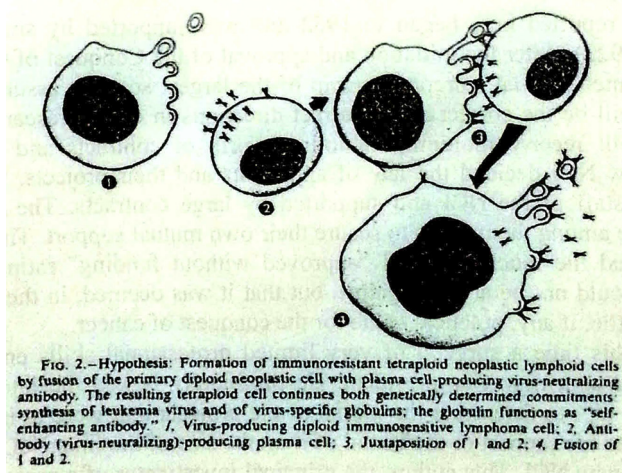


Fig. 1. The cover of the book *Leukemia-Lymphoma* containing the presentations of the 14th Annual Conference of Cancer at MD Anderson Hospital in 1969, where Sinkovics et al. presented his first report on his research projects including the first formation by fusion of a spontaneous tetraploid cell, which was the first ‘hybridoma’ producer of monoclonal antibodies (Fig. 1); the first appearance of large granular human lymphoid cells that have become “Burnet’s immune surveillance cells” and later natural killer cells with cytoplasmic granules (Fig 6), different from the already known T lymphocytes (Fig 21); and episodes of human lymphocyte-mediated cytotoxicity. (Monocytoid cells by mistake were printed as “macrophagic”). Sinkovics JG Shirato E Gyorkey F Cabiness JB Howe CD Relationship between lymphoid neoplasms and immunologic functions. In *Leukemia-Lymphoma*. Year Book Medical Publishers Pp 53–92 1969–1970.

Note: Figure numbers refer to those of the cited article

still in Hungary at the Department of Virology of the State Institute of Public Health in the mid-1950s, one of us succeeded in isolating Newcastle disease virus (NDV) from women with benign reversible conjunctivitis, while they were preparing chicken meals. This virus grew in the brain of suckling mice and killed Ehrlich ascites cancer cells in adult mice but lost its pathogenicity in baby chicken. The Hungarian anti-Soviet uprising in 1956 ended (for a while) our experimentations and delayed their publication over a

year. We have never seen a citation of our original articles published by Doerr & Hallauer in the *Archives fur die gesamte Virusforschung* (7:242–257;1957;7:403–411;1957), while dozens of papers appeared on oncolytic viruses elsewhere. The PR8 (Puerto Rico) attenuated influenza virus became our first oncolytic virus in the USA, lasting until our friendship and cooperation with Bill Cassel began, who let us change it to his NDV in the 1980s (*Acta Microbiol Immunol Hung* 53:367–429:2006). An armada of extraordinary co-authors made our monograph a worldwide bestseller [1].

ABBREVIATED REFERENCES ORGANIZED

Sinkovics’ (JGS’) monograph [2] in its 2,370 references includes over 73 citations of Sinkovics et al. [3]. Sinkovics’ large granular lymphoid cells discovered first in the human blood (in his own blood) as “Burnet’s Immune Surveillance Cells” (1969) are identical with the late-designation “Natural Killer Cells” (1975) and are historically cited (*Ann Clin Lab Sci* 49:691–698;2019; *Int J Virol AIDS* 2020. 7:072 volume 7 issue 30) and [4].

Ancient original articles of historical value that introduced viral oncolysis, are no more cited. These are on the human pathogenicity of the NDV and its relation to the mumps, fowl plague and influenza viruses. The first studies were on the biological characteristics of the oncolytic (to Ehrlich ascites carcinoma cells) NDV adapted to the brain of newborn mice (*Archives gesamte Virusforschung* 7:242–257;1957;7:403–411;1957;10:103–125;1960). The publication of these manuscripts was delayed by Khrushchev’s tanks mobilized to crush the Hungarian uprising against the Soviet Union in 1956.

WORKING AT THE YOUNG MD ANDERSON HOSPITAL

The Departments of Medicine and Hematology at MD Anderson Hospital were directed by professors Clifton Dexter Howe and C Charles Shullenberger. One of us (JGS) served from the rank of resident fellow in 1958 to full professor and Chief of the Section of Clinical Tumor Virology and Immunology until 1979. His prominent clinical associates were assistant-associate professors MDs Nicholas Papadopoulos and Carl Plager. His prominent clinical research assistants/associates were numerous basic scientists and PhDs, who contributed to, thus co-authored relevant articles: the exemplary H David Kay PhD. Of the numerous assistants, Glenda Groves and Barbara Bertin carried out basic research on the mouse leukemia virus terrain [5]. At the end (1979), Jim Romero stood out in the clinical application of a human viral oncolysate vaccine (*Internat J Virology AIDS* 2019, 6:049 vol 6 issue 1). Popular reprints of the *Adult Human Sarcomas I-II* had to be reprinted (*Expert Reviews Anticancer Therapy* 7(1,2),31–56:183–210;2007).

Thereafter Sinkovics was invited to and accepted the leadership of Tampa's St. Joseph's Hospital's new cancer center (institute) from 1983–2006. Sister Marie Celeste Sullivan elevated Tampa's St. Joseph's Hospital above the best by emphasizing the need for clinical (and laboratory) cancer research. Joseph C Horvath from Canada (originally from Semmelweis University, Budapest, Hungary) associated with Sinkovics for the laboratory production of the patients' various immune lymphocyte preparations and Cassel's Newcastle disease virus oncolysate vaccine administered by State of Florida licenses (as summarized in Sinkovics' My Life My CV) [4]. Our laboratory benefited from expert advices of chief pathologist university professor Jenő Szakacs; in his retirement he was invited to serve as a Moffitt's professor of pathology. He, Ferenc Gyorkey and Sinkovics shared extraordinary interest in the viewed, repeatedly observed but never cultured "human sarcoma retroviruses" related to the avian Rous sarcoma virus and the Moloney murine sarcoma virus (Szakacs JE, Szakacs MR. *Ann Clin Lab Sci* 5:14–22;1975; Sinkovics JG, Gyorkey F. *Cancer* 27,1449–1459;1971). Unfortunately, Frank (Dick) Rauscher's National Cancer Institute failed to recognize the academic rise of Sinkovics et al. (under the malicious influence of one particular project site visitor who failed to recognize the new "cytotoxic large granular lymphocytes", later NK cells, in her own material, thus tried to deny their existence).

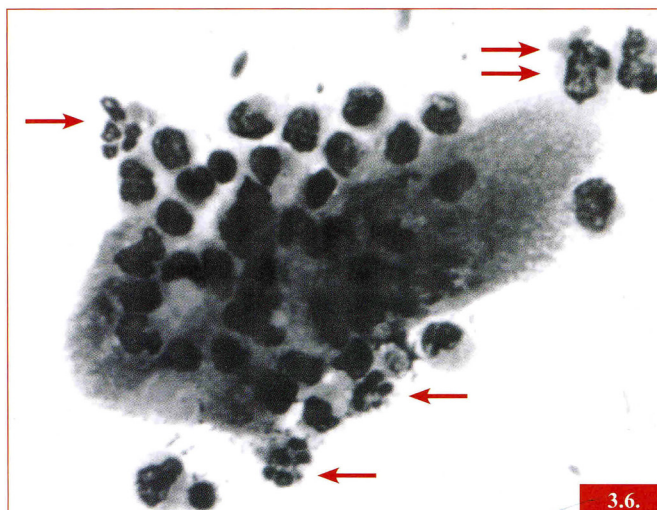
TUMOR CELLS DESTROY ATTACKER LYMPHOCYTES

The scenery of lymphocytes killing tumor cells has been documented frequently (Figures) [2, 3], whereas opposite events in which tumor cells killed attacker lymphocytes were

rarely seen, thus remained unexplained, especially when the tumor cells remained preserved, viable and morphologically intact. The molecular mediators involved in this interaction remain so far inadequately identified.

We imagined the ancient Earth where the initial RNA/DNA complex ruled the development of the primordial cells. Here, life forms displayed into what developmental stages the modern RNA/DNA complex could periodically regress [3, 6–8]: the natural phenomenon of the "retrograde cellular immortalization", disorderly but immortal. The clinics diagnose "carcinogenesis": a natural stage of the evolving RNA/DNA complex [3]. The immune system of the host either accommodates or destroys it.

Here (Figs 2 and 3) tumor cells release invisible mediator molecules for the destruction of tumor cell-killer lymphocytes, thus for the self-defense of the tumor cell and basically for the protection of the tumor against the host. Tumor cell-attacker host lymphocytes are induced to undergo "nuclear clumping" (later called: 'apoptosis') and death. This process works for the self-defensive protection of the tumor cell, which thus escapes lymphocyte-mediated cytolysis. Originally, the Fas ligand to Fas receptor system regulated the process [2, 9] (fragmentation antigen system). Host lymphocytes released to attack the malignant cells are killed by the malignant cells for their defense, what is a turn against the host. The tumor is killing the host's anti-tumor lymphocytes by inducing their apoptosis (originally it was signaled as 'nuclear clumping'). This scenery opens up a field in which the attacker lymphocytes fail to eliminate the tumor; the tumor prevails. The process works for the self-defensive protection of the tumor cell. The defensive lymphocytes are released by the host in recognition of the malignant cells, for their destruction. The process is reversed as the tumor is killing the hosts' tumor-killer lymphocytes by



3.6. Death by nuclear clumping (arrows) of patient's lymphocytes (N. M. MDAH#73587) attacking an autologous human chondrosarcoma cell from cell line #1459 [2174], which withstand the attack without nuclear clumping or cytolysis. Erratum: this tumor cell was once referred to as "a soft issue sarcoma cell, like extra-osseous chondrosarcoma," [305], but was identified firmly as a chondrosarcoma cell from cell line #1459 [306; 928].

Fig. 2. B&W Scenario 1 MDAH patient #73587 with chondrosarcoma. Autologous lymphocytes from peripheral blood resembling T cells while attacking tumor cells in vitro die apoptotic death (nuclear clumping in the 1970s). Tumor cells from autologous cell line (not HeLa cells) remain intact in several days. Characteristics of attacking lymphocytes are those of T cells. In the right upper corner two arrows point at two large apoptotic cells that appear to be NK cells. Thus the chondrosarcoma cell kills not only T but also NK cells. This explains its extraordinary pathogenicity. Several lymphocytes undergo death in "nuclear clumping" (apoptosis)

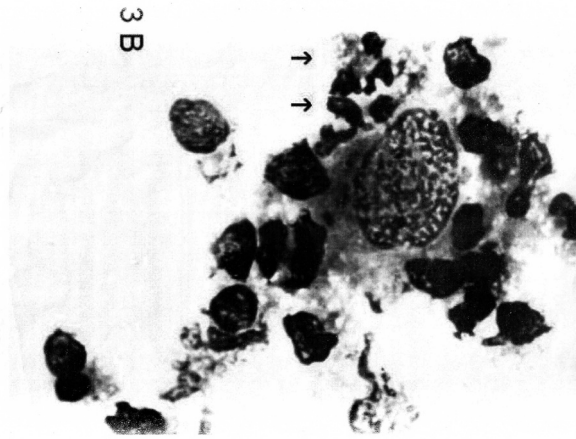


Fig. 3. B&W Scenario 2 Cultured dying myxofibrosarcoma cell of cartilage derivation with apoptotic nucleus is surrounded by large intact autologous granulated lymphocytes (“Burnet’s immune surveillance cells”, later NK cells); on its side (double arrows) apoptotic autologous small compact T cells assembled. Photograph preserved from the MD Anderson’s Sinkovics Lab from the early 1970s [2]. In tissue culture, the patient’s large round cells with the appearance of natural killer cells (formerly Burnet’s immune surveillance cells) surround an autologous dying tumor cell (a myxofibrosarcoma), which displays its apoptotic large nucleus. On its side (double arrows) deformed apoptotic immune T cells appear as they succumb. Preserved from the Sinkovics laboratory at MD Anderson Hospital in 1973. To be presented by Sinkovics JG, Thota H*, Kay HD, Loh KK, Williams DE, Howe CD, Shullenberger CC. Intensification of immunotherapy: attempts at measuring sarcoma specific reactions in vitro. Neoplasm Immunity: Theory and Application. Proceedings Chicago Symposium. Ray Crispen, Editor, Pp 137–152 1975. *to be presenter, deceased before the presentation

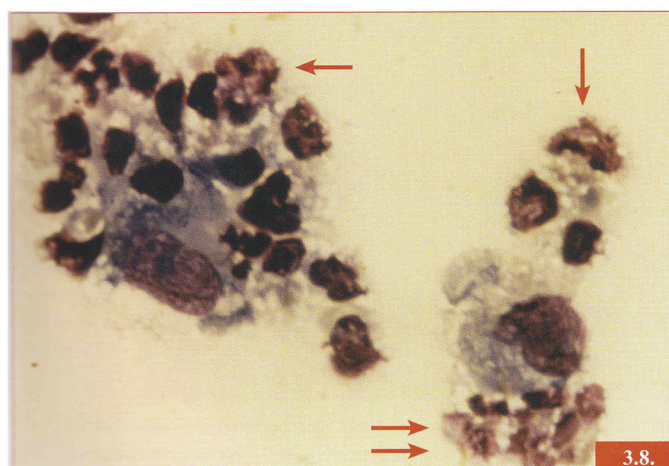
inducing their ‘nuclear clumping’ (by now: ‘apoptosis’). This scenery may open up a field favorable to the host in which the attacker lymphocytes directed specifically at the

tumor cells acquire resistance to apoptosis and survive. Such a possibility is surmised in the rare picture in which the tumor cell dies encircled in the ring of NK cells and immune T cells (double arrows) of Fig. 3. For decades, a subdivision of NK cells are suspected to acquire this faculty. Hereby killer cells act naturally, whereas immune T cells by specifically directed active immunization. Theoretically, virally fused dendritic cell and tumor cell products should function as the most effective anti-cancer vaccines [10] (Fig. 4).

SARCOMA

Sarcomata are malignant tumors of connective tissue origin including the so called soft tissues and bones of three germ-lined animals of the oceans, lakes, rivers, vast expanses of dry land and skies. The human myxofibrosarcomas are used as a most representative example of this entity, as they may appear in three different categories from slow growth latent (common), mediocre and acute high grade formations. Infectious variants of these tumors exist in birds (Rous sarcoma) and in murine species (Gross’ mouse leukemia; Moloney’s mouse sarcoma) caused by highly defined retroviruses. Human sarcomata occasionally yielded retroviral particles that were visualized in the electron microscope but without documented pathogenicity in serial cell cultures. Unicellular protozoa or early multicellular animals of the oceans (ctenophores, cnidarians, sponges; hydra) frequently host viral colonies that co-exist not like pathogens, but rather as essential parasites [11, 12].

The metabolism of the sarcomata (soft tissue mesenchymal tumors) preserved the ancient features of their ancestors among them susceptibility to exogenous viruses, some of them oncolytic. Of oncolytic viruses [1], vesicular stomatitis virus emerged with wide efficacy, however, synovial sarcoma cells SW989 exhibited complete resistance



3.8. Difficult to identify human sarcoma cells [254, 306, 928] preserve their tinctorial integrity under attack by a mixed population of small compact and large granular lymphocytes; some of the lymphocytes disintegrate (arrows) under counterattack by the sarcoma cell [110; 306]. Observed and photographed in the 1970s [110, 254, 305, 306, 928], but not recognized as apoptosis at that time (Table 4) (1.7). (Applied for permission to re-publish).

Fig. 4. Colored Scenario 3. Human sarcoma cells cultured in the early 70s in the MD Anderson’s Sinkovics Lab. Small compact T and large granular lymphocytes surround and react to autologous sarcoma cells [2]

to it. Single agent oncolytic viral therapy with talimogen laherparepvec (herpesvirus, Imlygic) or pexastimogene devacirepvec (vaccinia virus, Pexa-Vec) in a cost/success (remission induction) ratio have not appeared attractive. Imlygic dissolved some local melanoma tumors. In immune checkpoint blockade, in the SARC028 trial, the anti-PD1 pembrolizumab failed in patients with bone sarcomas but induced 7 “responses” in 40 patients with soft tissue sarcomas. In undifferentiated pleomorphic sarcoma, pembrolizumab induced two complete and seven partial responses in 40 patients. Nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) induced 6/38 responses including targeted leiomyosarcoma and myxofibrosarcoma. IL-2 and NK cells delivered in aerosol for osteosarcoma lung metastases showed therapeutic effect in patients. Patients with lung metastases of synovial sarcoma expressing cancer testis antigens NY-ESSO/SSX, MAGE experience prolonged survival. In the SARC028 trial, soft tissue sarcomas testing positive for immune checkpoint blockade (ICB) could respond with complete remission to pembrolizumab (anti-PD-1). In the Alliance A09140 trial, nivolumab (anti-PD-1) and ipilimumab-treated (anti-CTLA-4) patients gave a 6/38 response rate, that was below the chemotherapy rate, however notoriously chemotherapy-resistant tumors leiomyosarcoma, myxofibrosarcoma responded to the ICB combination. The doxorubicin and pembrolizumab combination did not increase the rate but doubled the duration of the response. A recognized feature of the host reaction is the prevention of the transition of M1 anti-cancer macrophages into tumor promoter M2 macrophages. In synovial sarcomas, overexpression of cancer testis antigens NY-ESO in [3] in Figure 34 pp 69, 620-1 etc. correlate with increased 5-years survival rates [3, 13].

The human natural killer (NK) cell ligand to sarcoma cell receptor contact studied in vitro is essential to the understanding of how sarcoma cells repel NK cells. At the College of Allopathic Medicine, Nova Southeastern University, Fort Lauderdale FL, fresh human sarcoma cells collected from patients were tested by the expression of their proliferation markers, the so called PCNA and DNAM-1 ligands CD112 and CD155 for interactions with genetically modified NK cells. Freshly obtained human sarcoma cells appeared to repel tumor infiltrating NK cells. Cytoplasmic degranulation occurred in NK cells upon contact with sarcoma cells. The aim of this research is the rendering of the sarcoma cell to be susceptible to the hosts NK cells and the NK cells to retain their virulence [14].

Radio-chemotherapy-treated metastatic human sarcomas (myxofibrosarcoma) in complete remission, invariably relapse [15].

ABBREVIATIONS

CTLA cytotoxic T lymphocyte associated protein
PCNA proliferating cell nuclear antigen

DNAM DNA accessory molecule
ligand CD112 nector
ligand CD155 poliovirus receptor

Founding resources: Nothing declared. See Sinkovics Ann Clin & Lab Sci 2019;49(6):691–8.

Conflict of interest: The authors declare no conflict of interest.

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