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# Granulocyte Therapy for Cancer: A Prospective Review

Dong Wang,<sup>\*A</sup> Wen-ying Wang,<sup>†</sup> Lei Ren<sup>\*</sup>

A powerful and complicated defense system exists within the cell and the whole body to protect against “irresponsible” cell growth and to deal harshly with most wannabe cancerous cells. However, occasionally the malfunction of one of these systems may result in an uncontrolled cell growth, which has been found taking the first step toward becoming carcinogenesis. Immune system is the most important defense system to protect the host from diseases. There is strong evidence showing that immune system also plays an essential role in providing significant protection against cancer. However, how the immune system works in defending and fighting against cancer and what is the exact molecular mechanisms of this process are still unknown. We herein review the historical change of the immune-associated cancer therapy and then provide evidence on the exploration of the relationship between the immune system and cancer disease. Moreover, an outline about the serendipity discovery of the Spontaneous Regression/Complete Resistant mice and the corresponding selective cancer killing mechanism of which is mediated entirely by leukocytes from the innate immunity is discussed. In addition, based on the outstanding curative effect on the animal model, a promising anti-tumor therapy method, Leukocyte Infusion Therapy, has been approved by FDA and is being preceded in conducting clinical trials. ■

\*:Department of Biomaterials, College of Materials, Xiamen University, Xiamen 361005, P.R. China

†:School of Foreign Languages, Yuxi Normal University, Yuxi 653100, P.R. China

Δ:Correspondence to: Dr. Dong Wang, No. 217 Lingfeng Building, 422 Siming South Rd., Xiamen, 361005, P.R. China  
Tel: +86 15960273670  
Fax: +86 0592 2183937  
Email:wangdongfirst@gmail.com

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IT is well known that cancer is predominately a disease of elder individuals, the human above their 50 years of age have a higher risk for cancer (1). It is also not uncommon that some people remain cancer-free into their 80s and 90s, even if they are regularly exposed to environmental carcinogens such as

air pollutants and heavy cigarette smoking etc. Like most of the epidemic diseases, they spread in a large area or among a large population especially in the winter. As reported that human mortality is much higher in winter than in summer (2), this phenomenon seems never changing since data have been recorded (3).

Immune system, an essential evolutionary protective composition of our surveillance mechanism, plays the foremost important role in protecting the host from attacking by different types of diseases, especially the infectious diseases. The immunity seems vary with the seasons, which means people needs limited activity and more sleep to compensate the weaker immunity in the winter. In addition, there is strong evidence showing that a weakened immune

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system will increase one's chances of getting blood-cell and virus-associated cancer (4).

### From spontaneous regression of cancer to cancer immune surveillance

What is the exact relationship between immune system and cancerous diseases? This is a frequently asked question but without a clear answer. We herein briefly review the development of its history and give some typical events about the question.

From the historical records, the spontaneous regression of cancer is a well-documented, even rare, event for many types of cancer (5, 6). While great efforts have been taken over the past decades, the mechanisms of spontaneous regression have remained elusive. However, one interesting thing is that many reported cases of spontaneous regression of malignancy occurred along with infection (7, 8).

In the 1890s, William Coley, a New York pioneer surgeon, began exploring the immunotherapeutic approach to cancer. Coley developed a vaccine, now referred to as "Coley's toxins", consisting of extracts of killed gram-positive streptococcus pyogenes and gram-negative *Serratia marcescens*, can evoke many symptoms of bacterial infection such as fever and chills without the actual infectious problems. Tumors, especially the soft-tissue sarcomas and lymphosarcomas or lymphomas, were observed to partially or completely be regressed following treatment with Coley's toxins (9).

Although the reasons why sarcomas responded preferentially to the treatment of Coley's toxins are unanswered, it has been suggested that sarcomas have a higher probability of being immunogenic (10). The application of Coley's toxins in clinical practice eventually stopped due to his death and some inconsistent results, whereas Coley's efforts to stimulate the immune system to combat cancer

credited him being as the father of today's cancer immunotherapy.

In 1909, Paul Ehrlich, the 1908 Nobel Prize laureate in Physiology and Medicine, suggested that cancer and the functional capacity of an individual's immune system are interdependent. He proposed that in a normal human body there existed cancer cells, but an inherent system also existed that could identify and remove cancer cells before they accumulate into detectable cancer lesion in the absence of external therapeutic intervention. After then, Ehrlich named his hypothesis as "cancer surveillance" (11), which means the existence of few cancer cells in normal human body may not threaten daily health only if the growth of cancer cell and the cancer surveillance are in the dynamic balance of supersession and remodeling.

The hypothesis of tumor immune surveillance was then formalized in the 1950s by Sir MacFarlane Burnet, the 1960 Nobel Prize laureate in Physiology and Medicine, and Lewis Thomas on the basis of the advanced mechanisms of the role of cellular immunity in eliminating allergenic or altered cells including cancerous cells. They then hypothesized that lymphocytes were the critical component for recognizing and eliminating the continuously arising and newly transformed cancerous cell. (12). However, the theory of tumor immune surveillance subsequently remained controversial and found difficult to be demonstrated experimentally. The newly developed basic and clinical evidence supported the significant effect of immune system, mainly the lymphocytes, on the cancer immune surveillance, but the potential contribution of other leukocyte populations was largely unknown.

For the existence of cancer immune surveillance, Chester Southam provided another piece of evidence (13). Southam injected a tumor cell line (Hela cells) into humans who were divided into two groups. The study showed that non-cancerous patients rejected the tumor cells; in con-

trast, subjects with advanced cancer were able to sustain tumor growth for a considerably longer period of time from the cancerous transplants, which suggested that the later had impaired immune function against cancer. Moreover, the impaired immune function was not resulted from the immunosuppression, as the production of antibodies against viral infection was found to be the same in non-cancer patients as those with cancer. The study discussed and argued that the decline in immune function was cancer specific. Otherwise, there naturally exists an immune surveillance system in some people's body, and this surveillance is cancer specific. However, it does not exist in all people's body.

### A serendipity discovery of the Spontaneous Regression/Complete Resistant (SR/CR) super mice

The exploration of the relation between the immune system and cancerous diseases continued, and 50 years later, a serendipity discovery and a series of research work put this hypothesis on spot again. (14)

In 1999, Dr. Cui and colleagues injected mice with an ultra-virulent ascites carcinoma cells, Sarcoma 180 (S180), during a routine experiment designed to generate ascites for enhancing antibody production. Unexpectedly, one male mouse survived with S180 and remained ascites free after repeated injection. S180 is a historically wide-used mouse cancerous cell line that was originally derived from the connective tissue of Swiss mice and is reported to express negligible levels of Major Histocompatibility Complex (MHC) surface proteins, thus enabling it to demonstrate unrestricted growth within several different genetic strains of mice. Due to its aggressive nature, no reports there regarding the resistance to S180 induced ascites. After then, confirmation studies were carried out and found the original mouse survived after increasing the does up to

$2 \times 10^9$  S180 and remained ascites free.

Since then thousands of mice in about 50 generations have been bred from the original cancer-free super mouse and approximately 30 percent of the offsprings have inherited the cancer resistant trait in an autosomal manner.

These mice strain were named later by Dr. Cui as Spontaneous Regression/Complete Resistant (SR/CR) mice. The purpose in naming the mice SR/CR was due to the age-dependent responses witnessed at the time of cancer screening. Among offsprings that eventually showed to be SR/CR, those that are screened at 6 weeks of age quickly demonstrate complete resistance (CR), whereas approximately half that go on to be initially screened at 12 weeks of age would develop S180-induced ascites before demonstrating spontaneous regression (SR).

Further studies in Dr. Cui's lab have shown that the cancer resistance in SR/CR mice is mediated by the special immune system, exactly as the innate immune system with neutrophils, macrophages, and natural killer cell among the most abundant responding cell types (15).

What is the evidence about the innate immune system, rather than adaptive immune system or others, in response to the action unit? Before discussing the novel discovery and the scientific explanation, we will first present some basic concepts of the immune system.

The innate immune system, also known as non-specific immune system, is capable of providing an immediate first line defense over the host from infection by external organisms through recognizing common features of pathogens in a non-specific manner. In contrast, the adaptive immune system permits the recognition of novel pathogens through the clonal selection of antigen-specific receptors. The adaptive response is retained after elimination

of the pathogen in the form of immunological memory, which provides enhanced levels of protection from subsequent re-infection from the same pathogen. The innate immune system plays an important role in both the activation of the adaptive immune system and the control of infections before the initial adaptive immune response taking effect (16).

The resistance against cancer mediated by innate immunity is supported by a lot of evidenced facts as fol-

...What an amazing unbelievable scene that the elder cancer resistant mouse developed ascites like the other commons and then the ascites disappeared suddenly overnight at about 15th day after the cancer cell challenged...

lows (15, 16): (i) The SR/CR mice survived the initial challenge with cancer cells without any prior exposure suggests that the anticancer response is innate immunity, and the innate immune cells remain as the major responding effector cells during repeated cancer cell challenges. (ii) The rupture of cancer cells by leukocyte subsets was clearly, visually characterized by transmission electron microscopy and immunofluorescence microscopy (17). On this video, the yellow arrow pointed out that granulocyte is killing cervical cancer cells. For this process, it was shown that some rosettes was formed between leukocyte subsets and cancer cells, and the composition in the rosettes was identified by using flow cytometry and surface marker labeled to indentify polymorpho-nuclear leukocytes (PMN, 38%), natural killer cells (NK, 35%), and macrophages (26%). Formation of rosettes between leukocytes and cancer cells is a specific property of the anticancer response in SR/CR mice and has not been observed in wild type (WT) mice challenged with cancer cells. (iii) The independent depletion of any of

these sub-types cannot abolish cancer resistance, while the total immune depletion resulted in tumor formation which could be reversed when the immune system was allowed to recover. (iv) Half of the offspring crossed from SR/CR mice and the nude mice in which the maturation of T lymphocyte is blocked by the absence of a thymus show the similar ascites resistance phenotype to the SR/CR mice. (v) Highly purified NK cells, macrophages, and neutrophils from the SR/CR mice independently kill cancerous cells in vitro. Besides, the purified populations of SR/CR macrophages have been shown can be transferred to WT mice to offer protection from S180 tumor formation. Thus it is clear that in the SR/CR mouse model, the innate immune cells, especially the neutrophils, are capable of mediating cancer immune surveillance.

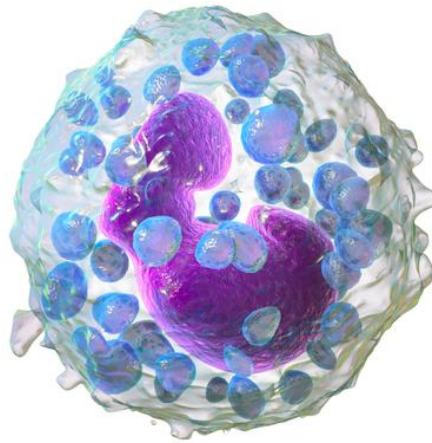
The innate immune response of SR/CR mice to S180 occurs in three distinct phases: infiltration, tight contact and tumor destruction (15, 16). First, upon challenges with cancerous cells, predominant innate host leukocytes can detect the unique signals of cancerous cells and migrate directly to the cancer site in response to the reversed chemical concentration gradient. The varieties of protein pieces specifically produced by cancerous cell may mainly be the chemo-attractants play a role in this process. In addition to S180, SR/CR mice were found to be resistant to EL-4 lymphoma, MethA sarcoma, P851 mastocytoma, and LL2 lung carcinoma etc, although there was a large difference in the maximum tolerated dose (MTD) to different types of tumor cells. The tumor cells with intense chemotaxis to attract leukocytes have a higher MTD, which leads to an escape from resistance. These results indicated that the chemo-attractants of tumorous cells and the chemotaxis function of leukocytes together play a pivotal role in the process of cancer killing (18). Second,

once they arrive at the sites of cancer, the host leukocytes tightly contact with the cancerous cells via surface recognition, of which is mediated by the cellular membrane charge difference between them. The surface recognition by leukocytes results in a suspended cellular aggregate and then formed the rosettes. The presence of the rosette provides a crucial experimental means showing that direct cell contact is essential for leukocyte-mediated cancer killing in SR/CR mice (18). Third, once they are engaged with cancerous cells, leukocytes undergo rapid transformation into effector cells, in which the effector molecules such as cytotoxic granules and perforin, and other cytolytic effectors like superoxides, and nitric oxides are generated. Such effector molecules cause damage to the plasma membranes swelling and eventual rupture of cancerous cells through both cytolysis and apoptosis pathways (18). The third process may not unique to SR/CR mice since these effector mechanisms have been reported previously in WT mice in killing the cancerous cells (18). However, the first two processes were unique to SR/CR mice since WT leukocytes are unable to respond to the same cancerous cells with either infiltration or rosette formation. Research on elucidating the exact mechanism of SR/CR resistance is still ongoing.

Another intriguing property of the resistance of SR/CR mice is that they are healthy even in an extended lifespan suggesting that normal cells in these mice are not harmed by the anticancer response that only targets on cancerous cells with exceptional high specificity. The cancer resistant phenotype can be retained for life if the mice are frequently challenged with cancerous cells (16).

### Leukocyte infusion therapy for cancer

The exact mechanism and the roles of innate and adaptive immune system in SR/CR resistance are not so clear. However, the therapeutic potential



for cancer is apparent and absolutely exciting.

Granulocytes are the most abundant type of white blood cells that account for as much as 60% of total circulating white blood cells in healthy people, and up to 95% of granulocytes are neutrophils. Donors can give granulocytes specifically without losing other components of blood through a process called leukapheresis that separates granulocytes and returns other blood components back to donors. This is an established procedure that has been in use for more than 40 years with very good safety records. According to the FDA regulation, a healthy donor can give granulocytes by apheresis blood donation 24 times a year.

To normalize the cancer resistance ability, an *in vitro* assay of Cancer Killing Activity (CKA) has been developed by Dr. Cui's research team (19). The CKA was normalized as percentages of total target cells during selected periods of incubation time and at selected effector/target cell ratios in comparison to non-effector cell controls. The CKA assay was initially used in SR/CR mouse phenotype test, and then investigated successfully in human leukocytes. The existence of CKA in humans is an initial step to the goal that can translate the anti-cancer therapeutic effect into human being.

In a human study with small sample volunteers, Dr. Cui and colleagues found that the cancer-killing activity of the granulocytes was in the highest level in people under the age 50. One of the unexpected dis-

coveries is that the CKA in some volunteers is higher than that in the SR/CR mice, although it is in the same low level in some people as in the WT mice. They also found that this activity can be decreased by factors like winter weather or emotional stress (19). The research raised a prospect that cancer-killing immune cells from donors can be used to significantly boost the cancer-killing ability of cancer patients to fight their diseases until cure them.

In the study, volunteers who are selected as donors, based on the observed potential CKA of their white blood cells, will complete the leukapheresis blood donation to collect their "super strength" granulocytes. The cancer patients will then receive the granulocytes through a transfusion, a safe process that is often used for patients who have antibiotic-resistant infectious diseases for many years. Dr. Cui named the therapy Leukocyte Infusion Therapy (LIFT). The concept makes us think again about our preconceived notions that how the immune system works against cancer. The clinical trial of Leukocyte Infusion Therapy has been approved by FDA with a ClinicalTrials.gov Identifier of NCT00003243 and is preceded in South Florida Bone Marrow/Stem Cell Transplant Institute (20). The study was to see if simple blood transfusions can transfer cancer immunity from people with strong cancer immunity to those without such strong immunity.

The key to the success for such a new therapy is to transfuse sufficient granulocytes from healthy donors while their cancer-killing activities are at the peak level. It is important that the donors and recipients are unrelated and the human leukocyte antigen (HLA) mismatched to avoid the possibility of transfusion-associated graft versus host diseases.

How can we collect sufficient "super strength" cancer resistance granulocytes? Whether patients can tolerate the sufficient amount of transfused granulocytes for the treat-

ment? Whether the treatment can result in significant clinical benefits for the patients? Which types of cancer are suitable for such treatment? All these questions are needed to be investigated by future studies.

In summary, cancer immune surveillance is a long historical but controversial topic in the progress of anti-cancer area. The fast-developing basic and clinical researches have been being focused on the effect of immune system, particularly the adaptive immune system. Regarding the cancer immune surveillance and cancer killing activity, the potential contribution of the innate immune system was largely unknown. The discovery of the SR/CR mice and the cancer killing mechanisms of which is mediated entirely by leukocytes from the innate immunity provides a powerful evidence for the significant role of the innate immune system in defending against cancer. Although the exact genetic and molecular mechanisms of the SR/CR “super mouse” family are still unclear, the therapeutic potential of LIFT for cancer are absolutely promising, not only for the clinical, but also for the fundamental knowledge about the relationship between the immune system and cancerous diseases. ■

### Conflict of Interests

None

### Abbreviations

SR: Spontaneous Regression; CR: Complete Resistant; WT: wild type; S180: Sarcoma 180; MHC: Major Histocompatibility Complex; PMN: polymorphonuclear leukocytes; NK: natural killer cells; MTD: maximum

tolerated dose; CKA: Cancer Killing Activity; LITF: Leukocyte Infusion Therapy; HLA: human leukocyte antigen

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