What are the main aims of your research?

I was intrigued by the power of an evolutionarily refined immune system in protecting humans and other life forms on Earth against diseases that have been occurring for millions of years. Since the earliest reported observation of an enlarged lymph node due to leukoreticular infiltrates in gastrointestinal malignancy by Rudolf Virchow in 1854, the immune system’s protective surveillance and tumour-promoting nature have perplexed investigators. Convinced by the important role of the immune system in cancer, I decided to devote my career to defining the requirements for effective anti-tumour responses by immune effector cells. In this way, I believe I can help discover how to tap into the power of the body’s own immune cells to combat cancer.

Your early observations into mouse tumour models and HIV-infected patients suggested that the immune system could play a crucial role in restricting cancer. Could you elaborate on the significance of this finding?

My doctoral work showed that growth of an aggressive lymphoma in mice was associated with depletion of thymocytes, the precursors to T lymphocytes. This resulted in immunosuppression in mice, which I reported in a number of scientific journals in 1999 including Tumor Biology, FEMS Immunology and Medical Microbiology. Also, during my brief tenure at the Division of AIDS of the Indian National Institute of Communicable Diseases (now the National Center for Disease Control), I observed an increased incidence of malignancies in HIV-infected patients. These findings suggested to me that a breakdown of immune surveillance could be the possible aetiology of cancer, which drove me to learn more in this field.

Can you explain how this finding led to your current research project?

My early studies clearly indicated that host immunity is well integrated with cancer development, progression and rejection. Thus, I felt that a deeper investigation into the complex mechanisms of immune function was certainly warranted. I discovered an indispensable crosstalk between tumour-reactive T lymphocytes (T cells) and natural killer (NK) cells that was reported in 2007 in the Journal of Immunology.

How do NK cells influence cancer therapy?

When NK cells are in the vicinity of active T cells at a tumour site, they help contribute to cancer therapy by rejecting or eradicating cancer cells that have lost their expression of cognate tumour antigen and have managed to evade their rejection by T cells. Natural killers are able to keep such antigen-escape variants of cancer cells at bay, which would otherwise metastasise and become lethal.

Why is it necessary to establish effective cancer therapies with no possibility for autoimmune reaction?

Immune therapies for cancer attempt to harness the immune mechanisms that eradicate tumours. Essentially, they are focused on breaking the body’s immune tolerance. However, in addition to their anti-tumour effects these therapies can also have bystander effects, meaning they can elicit autoimmune reactions by either antigen-non-specific or antigen-specific mechanisms.

Recognition of specific immunodominant antigens on tumour cells are desirable for cancer therapy because they can be targeted to trigger strong anti-tumour response with minimal autoimmune reaction. Unfortunately, such antigens are few and far between, and intensive research is ongoing to identify antigens that are appropriate for cancer therapy. One promising target is cancer-germline antigens due to their restricted expression in germinal cells with nominal tissue-specific autoimmune response.

Which of your research discoveries do you consider to be the most significant in the ongoing hunt for a cure for cancer?

I am especially pleased with the discovery that activated cytolytic CD8 T cells reacting to a self-tumour antigen provide a necessary ‘helping hand’ to otherwise dormant NK cells in eliciting their anti-tumour effector function. This finding is very significant, it establishes a new paradigm that the adaptive immune effectors can contribute to the activation of innate immune cells, advancing the field of basic and translational immunology. This is particularly important in light of our recent data that suggest that the T cell-NK cell functional crosstalk has the potential of being modulated and refined by therapeutic drugs.
Crosstalking immune cells

By learning more about the inner workings of T lymphocytes and natural killer cells, researchers at the Shanker laboratory at Meharry Medical College School of Medicine have revealed new insights into the body’s immunity to cancer.

IN THE WORLD of cancer research, what might at first appear to be a small discovery can often translate into novel therapies and treatments later on. One area benefiting from dedicated research is cancer immunotherapy, in particular the relationships that occur between cells and the triggers for cell mutations. Dr Anil Shanker, Associate Professor of Biochemistry and Cancer Biology at Meharry Medical College School of Medicine, is excited by recent data gathered by his laboratory, which shed new light on immunity and the cellular teamwork responsible for increasing anti-tumour immunity levels. Specifically, the team’s findings suggest that through the use of therapeutic drugs there is potential to optimise and refine functional crosstalk between T lymphocytes (T cells) – which perform a key function in cell-mediated immunity – and natural killer (NK) cells – a type of lymphocyte considered to be capable of binding tumour and virus-infected cells without external stimulus. It is observed that these two lymphocytes can work together to effectively eradicate solid cancers.

LYMPHOCYTE COOPERATIVITY

The central focus of Shanker’s work is to construct a clearer picture of the mechanisms driving T cell–NK cell crosstalk. This follows on from pioneering research he completed developing a solid tumour model able to demonstrate activated CD8+ T cells (a cytotoxic T cell that kills cancer cells) providing support to dormant NK cells in eliciting their anti-tumour effector function. "Various projects we are working on are either digging deeper into the mechanisms of this lymphocyte crosstalk or enhancing it with various immunomodulators such as the proteasome inhibitor bortezomib or pharmacological Notch ligands,” he explains.

Shanker has broad experience in tumour immunology, with training and expertise in lymphocyte biology. His background has provided the Meharry Medical College team with an excellent context from which to develop their tumour immunology and immunotherapy studies.

CROSSTALK MECHANISMS

T cells can help stimulate innate natural killers from their dormant state to fully functional effector state. They do this by providing some activation signals. However, the processes by which they achieve this are not well understood. "We have clear and direct evidence that activated T cells help stimulate dormant NK cells, but the evidence whether T cells help the recruitment or proliferation of NK cells is rudimentary and indirect,” observes Shanker. He is looking into these lymphocyte processes by various cutting-edge cellular and molecular imaging tools.

He and his team’s research, in collaboration with Dr Gregory Verdeil, University of Lausanne, Switzerland, clearly indicates that tumour antigen-reactive CD8+ T cells are able to transform the naive status of tumour-infiltrating NK cells to a killer effector phenotype at the site of tumour. This is why the anti-tumour crosstalk between CD8+ T cells and NK cells is of interest. The research has involved generating and characterising new transgenic mice that express T cell receptors (TCRs) specific to an unmuted self-tumour antigen. The researchers believe such antigens present promising targets for potential cancer treatments because they have a restricted expression in germinal cells and thus no possibility of global autoimmune reaction.

Some of the laboratory’s more recent studies involve further exploring the complex intracellular dynamics following interactions between T cells and NK cells. Their research employed a lymphocyte interaction co-culture model, which indicated that in a normal naive situation T cells rarely interacted with NK cells, but that in an activated state their interactions significantly increased. "Alterations in phosphorylation status of multiple signalling proteins during CD8+ T–NK interaction suggest a cellular remodelling, whereby NK cells polarise activated CD8+ T cells towards a central-memory phenotype and activated CD8+ T lymphocytes induce naive NK cells towards effector/regulatory phenotype,” Shanker reveals. Essentially, the next stage of work is to unravel the mechanisms behind this immune cooperativity. Shanker has teamed up with Dr Alla Ivanova at Yale University, USA, to dissect these mechanisms at the level of intracellular mitochondrial dynamics.

PROMOTING IMMUNE CROSSTALK BY THERAPEUTIC DRUGS

The newly-discovered facet of lymphocyte crosstalk presents an exciting opportunity to develop cancer therapies targeted at the tumour site that enhance functional cooperativity between immune cells. The focus of Shanker’s recent experiments has been on immunomodulatory drugs, in particular, the cancer therapeutic drug bortezomib that sensitises solid tumour cells to apoptosis. This
FUNCTIONAL CROSSTALK BETWEEN INNATE AND ADAPTIVE IMMUNE CELLS

OBJECTIVES
- To understand molecular mechanisms of functional crosstalk between innate and adaptive immune cells in cancer
- To identify immunotherapeutic modalities that can maximise anti-tumour cooperativity of lymphocytes for relapse-free cancer management

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has been reported in 2008 in his publication in the Journal of the National Cancer Institute. His work uses mouse models of kidney, breast and lung cancers to explore the way that bortezomib impacts lymphocyte responses in adaptive T cell transfer settings. “These studies demonstrate that, with an appropriate scheduling and dosing, it is possible to combine bortezomib with adoptive T cell and NK cell immunotherapy for the treatment of solid tumours,” he expounds. While each of the bortezomib, T cell and NK cell components are already in use in therapeutic practice, the team’s recent work studying combinatorial immunotherapy regimen represents a paradigm shift in the understanding of inhibitors grouped with immunotherapy and incorporation of these into clinical use.

NOTCH SIGNALLING
Another focus for the researchers is the significance of the Notch signalling pathway, where a transmembrane family of receptors regulates a variety of decisions at multiple stages of cellular development and differentiation. The team is interested to learn more about Notch signalling, which is targeted by cancer as a mechanism of evading immune response. This work builds on the results of a collaborative study published in 2011 in Cancer Research that showed that intact Notch signalling in lymphocytes is critical for the effective eradication of tumours as well as supporting remission in cancer patients. “Recently, we found that bortezomib can reverse Notch dysfunction in CD8+ T cells,” Shanker adds.

Laboratory work in collaboration with Drs David Carbone and Mikhail Dikov at The Ohio State University used mouse models to show that this process enhanced the effector function of lymphocytes against tumour cells, and led to the development of an increased pool of memory T cells. “Currently, we are attempting to achieve a molecular understanding of the Notch signalling network involved in anti-tumour T cell immunity,” notes Shanker. “We are assessing the therapeutic and prognostic potential of its modulation for translation into clinically relevant cancer therapeutics.”

Shanker has shown that intratumoral NK cells can be activated by surrounding T cells when they are responding to a tumour. This mechanism eliminates the development of tumour antigen-escape variants that can arise prior to metastasis. This two-way functional cooperativity between innate effectors and adaptive T cells, now apparent in various pathological and physiological settings of immune rejection, has broad implications for cancer immunotherapy.

Consensus has now evolved from analyses of large patient cohorts that common functional immune signatures exist to specify complete tumour regression. “These studies suggest a new paradigm in our understanding of T cell effectors in cancer biology and highlight the importance of designing clinical protocols that promote intratumoral T cell–NK cell functional cooperativity for successful therapy of cancer,” Shanker concludes.

Molecular targeting
Chemotherapeutic drugs or targeted inhibitors

Reduction of tumour angiogenesis and/or burden

Improve lymphocyte differentiation & function

Overcome immune suppression

Tumour eradication

T cell + NK cell transfers

Combinational immunotherapy for effective cancer treatments.