Recent Tools for Infection and Immunity Research

Helen K.W. Law
Centre for Human Immunology, Institut Pasteur, Paris, France.

The advancement of technology has expanded our knowledge in infection and immunity tremendously. In the past decade, the development of state-of-the-art flow cytometers combined with new fluorochromes and tandem dyes have allowed polychromatic analysis of cells. With careful design and optimisation of staining panels, it is possible to detect and enumerate very rare stem cells, cancer cells, virus infected cells and antigen presenting cells in the circulation. In addition, the use of flow cytometry for the study of signalling pathways (PhosFlow) and cytokine secretion (intracellular cytokine staining) have matured. New technologies have been developed beyond flow cytometry to increase the breadth and depth of cellular analysis. For example, using <50 microlitre of serum/medium, more than 50 cytokines can be assayed by multiple analytes profiling (xMAP technology) and single cell gene expression can be assayed at nanolitre scale (Fluidigm). Moreover, high content image analysis allows the study of immune synapse, granules/organelles, nuclear translocation, internalisation, and other cell morphology changes. It can also be used for genome wide siRNA screening. The latest wave is to study signal transduction pathways using quantitative mass cytometry which enable the concurrent measurement of 50-100 markers in thousands of cells at single cell level (CyTOF). The continual challenge would be to design methods for the analysis of huge data sets, to interpret the results, and, to decipher the complex relationships between pathogens, cells and molecules.

Advances in Molecular Pathology in Cancer and Inflammation: Role of TGF-Beta.

Hui-Yao Lan
Department of Medicine and Therapeutics, and Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

It is now well-recognized that inflammation can drive tumor progression. Transforming growth factor beta (TGF-β) may be an essential regulator in this process. Beyond the suppressive effect of TGF-β on the carcinogenesis, TGF-β promotes cancer progression through modification of both cancer stromal cell and carcinoma cell behavior and enhances their interaction within the tumor microenvironment. TGF-β is highly produced by cancer cells as well as stromal cells, which, in turn, alters the cancer microenvironment and promotes cancer cell growth, invasion, and metastasis via both autocrine and paracrine mechanisms. In the inflammation-associated cancer, TGF-β signaling (Smad2/3) is highly activated in both tumor and stromal tissues, which is associated with loss of an inhibitory Smad7 mediated by the Smad3-induced Smurf2-dependent ubiquitin degradation mechanism. Enhanced activation of TGF-β/Smad signaling, presumably Smad3, can promote cancer progression by inducing EMT, a key process in cancer stromal tissue remodeling and cancer cell migration and metastasis. Moreover, TGF-β is able to enhance cancer cell growth and metastasis by stimulating angiogenesis, which is mediated by Smad3-stimulated VEGF. In addition, we also found that Smad7 can inhibit NF-κB signaling by inducing IκBα, an inhibitor of NF-κB phosphorylation. Thus, loss of Smad7 in the cancer site may be attributed to the activation of the NF-κB signaling pathway through which cancer cell proliferation and inflammatory response are enhanced. Importantly, it is now clear that carcinoma-immune cell cross-talk initiated by TGF-β signaling is important in determining the tumor progression and metastasis. Increased TGF-β signaling contributes significantly to tumor-associated macrophage infiltration and induces T regulatory cell differentiation via the Smad3-depedent mechanism, resulting in cancer inflammation but blunting immune surveillance, favoring tumor growth, invasion, and metastasis by escaping the host immune defense system. All these studies suggest that the imbalance of TGF-β signaling such as over-activation of Smad2/3 associated with loss of Smad7 and dysregulation of TGF-β functions in the stromal-tumor axis may be critical in cancer growth, invasion, and metastasis. Thus, targeting TGF-β signaling in the stromal-tumor axis by overexpression of Smad7 may represent a novel and effective anti-cancer therapy.

Laboratory Investigations of Allergic Diseases

Eric Y.T. Chan
Division of Clinical Immunology, Department of Pathology and Clinical Biochemistry, Queen Mary Hospital, Hong Kong.

The incidence of allergic diseases in Hong Kong has increased several folds in the past 20 years. This phenomenon has been observed worldwide especially in developed countries. Allergy testing in identifying the causative allergen is therefore gaining importance. The results are applicable to clinical management in terms of avoidance of allergens and specific immunotherapy. With an appropriate clinical history several laboratory tests are helpful in determining an atopic status (total IgE) and finding the causative allergens. Atopy is the term referring to an IgE propensity. The causative allergens in IgE-mediated diseases can be determined in the laboratory by serum specific IgE and basophil activation tests. Non-IgE mediated allergic diseases are mediated by lymphocytes or non-IgE immunoglobulins. Lymphocyte proliferation or cytokine release assays are most often used laboratory tests to find out the responsible agents in cellular type (type IV) of allergy. Anaphylaxis is due to mast cell degranulation caused by specific IgE. Patients with anaphylaxis are often non-atopic. Mast cell degranulation can be confirmed in the laboratory by measuring the serum tryptase level. The causative agents are investigated by the methods described above.

Mechanisms Underlying HIV Evasion of Immunity and Their Contributions to Opportunistic Infections

Allan S.Y. Lau
Cytokine Biology Group, Department of Paediatrics and Adolescent Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong.

Human Immunodeficiency Virus (HIV) is the primary etiological agent for AIDS. Among the nine HIV-1 viral proteins, transactivator Tat functions as a key modulator in viral replication and acts as a potent immunomodulator in AIDS pathogenesis. The HIV Tat regulatory protein has been known to have multiple regulatory roles including the regulation of cytokine production and apoptosis. Its dysregulation of IL-6, IL-10 and TNF-α expression contributes to disease complications in AIDS including Kaposi's sarcoma, B-cell lymphoma and HIV-associated dementia. Recent reports have elucidated some of the mechanisms underlying how HIV-1 Tat induces cytokines through the activation of specific kinases and transcription factors. Additionally, HIV-1 Tat interacts with other cytokines to disrupt cellular function thereby contributing to HIV evasion of the immune system. For example, HIV-1 Tat perturbs IFN-γ receptor signalling and downregulates MHC presentation. Collectively, these cellular changes induced by HIV may be beneficial for pathogen evasion of immunity. Thus HIV has been shown to interact with opportunistic pathogens including Kaposi sarcoma-associated herpesvirus, protozoa, fungi, bacteria and mycobacteria in their effects on immunity. We recently showed that HIV-1 Tat also perturbs the Toll-like receptor system including the lipopolysaccharide activated signalling pathways. Taken together, these HIV-1 Tat-induced effects may provide a favourable milieu for the survival of HIV as well as the co-infecting pathogens, thereby contributing to the HIV-associated diseases. Therefore, anti-Tat treatment becomes one of the key areas for AIDS therapeutics development, with clinical trials of several drug candidates targeting at inhibiting Tat activities. In conclusion, understanding the mechanisms of action for HIV-1 Tat effects and their associated dysregulation of cytokines may provide new leads for the development of novel AIDS therapeutics. (Publications from A. Lau Group: Blood 2009, AIDS 2009 & 2010, J Leukoc Biol. 2010, J Immunol 2005. Supported by Research Grants Council and Research Fund for the Control of Infectious Diseases, Hong Kong.)