



Application of Immunological Methods to the Biology, Diagnosis and Treatment of Diseases

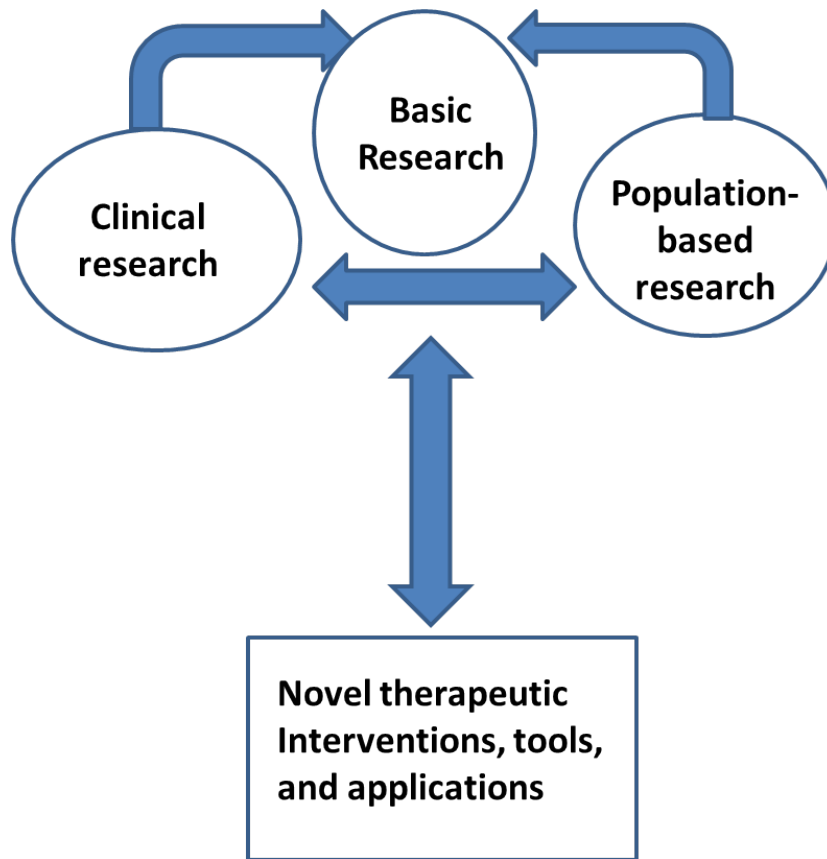
Overview: Immunology is defined as the branch of Biomedical Sciences concerned with all aspects of the immunological framework in all multicellular organisms. Modern immunological techniques at Cambridge Biomedical have a wide range of applicability, from Basic Science, Translational research to Clinical Application. Described here are some of the disease areas in which we have applied our considerable expertise in immunology.

Basic Science research includes investigations of the innate and acquired immune systems, cancer immunology, immunology of infectious diseases and intracellular signaling mechanisms used by cells of the immune system, among others. **Clinical Applications of Immunology** are diverse, and Immunologic methods are used in the treatment and prevention of large number of **immune**-mediated diseases. Recent advances in Diagnostic Immunology, such as miniaturization, amplified immunoassays, use of monoclonal antibodies in Flow cytometry and immunofluorescence, and incorporation of molecular biology principles in immunological methods, has revolutionized the applicability and scope of immunological methods in multiple therapeutic areas, including, but not limited to, the immunologic aspects of a very broad range of human diseases, encompassing investigations in the fields of cancer and cancer therapeutics; transplantation of solid organs and stem cells; autoimmune diseases including endocrine diseases such as Type 1 diabetes, rheumatologic diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE); pulmonary diseases including asthma and chronic obstructive pulmonary disease (COPD); neurologic diseases such as multiple sclerosis (MS); gastrointestinal diseases such as Crohn's disease, Celiac's Disease, and Ulcerative Colitis (IBD); and many others.

A rapidly growing field of immunological science is **Translational Immunology**, which is loosely defined as the branch of science that supports translating basic immunological discoveries into clinically useful tools, biomarkers and therapeutic interventions, and ultimately Standard of Care; this is also known as the 'bench-to-bedside' approach. A number of immunological techniques, such as Flow cytometry and multiplexed Luminex assays, are used to develop diagnostic and prognostic biomarkers and, ultimately, tools for immune-based interventions.



Flow-chart of Translational Research





Therapeutic Areas

Neurodegenerative Disease

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's Disease, is a progressive neurodegenerative disease affecting motor neurons in the brain and spinal cord, with a worldwide incidence of about 2 per 100 000 population and a prevalence of 4 per 100 000 population. Mesoscale Discovery (MSD) is a ligand-binding assay based on MULTI-ARRAY[®] technology, a proprietary combination of electrochemiluminescence (ECL) detection and patterned arrays. MSD assays provide a faster alternative to traditional ELISAs for performing sandwich immunoassays, and in many cases can be more sensitive and have a larger dynamic range than ELISA. The expression and prognostic relevance of pro-inflammatory Chemokines, Cytokines and credible Biomarkers of ALS are accurately assayed by MSD in cerebrospinal fluid (CSF) and plasma of patients diagnosed with ALS.

Hematological Malignancies

Acute and Chronic Leukemia; Hodgkin's lymphoma; Non-Hodgkins lymphoma, including B-cell, T cell and Natural Killer cell lymphomas and Waldenström's macroglobulinemia; and multiple myeloma, are examples of hematological malignancies of varying prognosis and survival rates. Diagnosis and stratification of acute leukemia into lymphoid and myeloid varieties is crucially important for treatment, and is routinely and accurately performed by Flow Cytometric immunophenotyping. Immunophenotypic classification by Flow Cytometry has distinct advantages over the limited classification by morphology alone, as distinct prognoses and clinical behavior are known to be associated with each Immunophenotypic sub-type.



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Flow cytometric immunophenotyping is useful in diagnosing lymphoma under the WHO classification system, where lymphoid neoplasms are separated into distinct clinical entities based upon morphology, immunophenotype, genetic abnormalities and clinical features¹. Prognostic Biomarkers are accurately studied by Flow Cytometry, which is also employed to classify lymphomas based on surface immunophenotyping; Fluorescence in situ hybridization (FISH) and quantitative PCRs are used to classify lymphomas based on cytogenetics.

Dysregulation of intra-cellular cell signaling pathways in hematological malignancies, as well as identification and lead compound stratification of small and large molecule compounds that impact the cell signaling pathways, is accurately performed by Flow Cytometric intra-nuclear phosphorylation studies.

Immunomonitoring

Immune monitoring assays include Microarrays, multiplex Luminex cytokine/chemokine assays, surface immunophenotyping by Flow Cytometry, phosphoepitope Flow Cytometry, and intracellular cytokine staining by Flow Cytometry. Immunomonitoring is applied in studying the impact of a biotherapeutic, small molecule compounds, large molecule (biologic) drugs, adoptive T cell therapies etc., on the immune system. These include the percentages and ratios of immune cells such as T and B cells; the induction of the short and long term memory phenotype; the activation state of effector immune cells, and the cytokine production and output of relevant cytokines and chemokines. These parameters can directly predict the outcome and impact of the therapeutic modality, as well as assist in diagnosing an adverse event, such as a cytokine storm.



Rare Diseases

Lysosomal storage disorders (LSDs), including various Mucopolysaccharidosis and Sphingolipidoses are a large group of more than 50 inherited disorders caused by a deficiency of specific enzymes responsible for the degradation of substances present in lysosomes, resulting in abnormal accumulation of undigested or partially digested macromolecules, which ultimately results in cellular dysfunction and clinical abnormalities. LSDs manifest as systemic diseases in patients with multiple and progressive neurological, renal, cardiovascular, gastro-intestinal, musculo-skeletal, ophthalmological, cutaneous and respiratory problems. While individual LSDs are rare, taken together as a group, they account for a sizeable disease burden, especially in certain ethnic populations, for example Gaucher's Disease in Ashkenazi Jewish population.

For many LSDs, early diagnosis and intervention before the onset of irreversible pathology will provide a substantial benefit to the newborn. Newborn screening, for example enzyme activities in dried blood spots, is important for early diagnosis of LSDs, especially in vulnerable groups.

Diagnosis of LSDs prior to enzyme replacement therapy is mainly based on detection of a specific enzymatic deficiency, as well as genetic testing. Monitoring of disease post-treatment is critical. Surrogate biomarkers have special significance in the monitoring of treatments for rare diseases, where the small patient sample size and heterogeneous expression of underlying pathology pose difficulties for direct study and where expensive treatment must be justified in terms of efficacy². Examples of surrogate biomarkers used currently include plasma and urine concentrations the principal storage product, Gb₃ in Fabry disease by Tandem Mass Spectrometry; and Urinary glycosaminoglycan (GAGs) concentrations by ELISA.

Paroxysmal nocturnal hemoglobinemia (PNH) is a rare hematopoietic stem cell disorder that arises from a mutation of the phosphatidylinositol glycan-class A (*PIG-A*) gene, which is located on the X chromosome. Hemolysis is a consequence of abnormal erythrocyte sensitivity to complement-mediated lysis, and hence, therapies that block the membrane attack complex are successful. Flow cytometric immunophenotyping is the gold standard for diagnosis, while monitoring of inhibitors of complement-mediated lysis done by ELISA.



Celiac's Disease

Celiac disease (CD) or gluten-sensitive enteropathy, is a chronic disorder of the digestive tract that results in an inability to tolerate gliadin, the alcohol-soluble fraction of gluten, resulting in Gastrointestinal (such as diarrhea) and Extra-intestinal symptoms. Patients with CD are reported to have antibodies against endomysium, reticulin, gliadin and tissue transglutaminase. Endomysial antibodies (EMA) of the IgA and IgG classes are detected by indirect immunofluorescence, and positive sera are then further classified based on serum titers of these antibodies. EMA of the IgA class are the most sensitive and specific markers. EMA of the IgG class also occur when IgA class EMA are in high titer or in individuals who are IgA deficient. These antibodies are also employed for monitoring the adherence of CD patients to a gluten-free diet and also to assay the effectiveness to other treatment modalities.

Diabetes Mellitus

As defined by the American Diabetes Association, Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories: In one category, type 1 Diabetes, caused by an absolute deficiency of insulin secretion; and the more common type 2 Diabetes, caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Both have short-term and long term consequences caused mainly by hyperglycemia. Current diagnosis is by retinopathy with fundus photography and measured glycemia as fasting plasma glucose (FPG), 2-h PG (2-h values in the oral glucose tolerance test), and Glycated hemoglobin A1C . The A1C test provides information about a person's average levels of blood glucose over the past 3 months, and is performed via ELISA. It is an indicator of how effective the management of diabetes is in a patient.



Oncology

Multiplexed bead-based Luminex assays are used for elucidating changes in cytokines, chemokines, and other biomarkers after treatment of various malignancies, such as Glioblastoma multiforme (GBM), Ovarian and Hepatic Carcinomas, Sarcomas, Lung Carcinomas, and various hematological malignancies. The Luminex assays can aid in the diagnosis of Cytokine storms, as an example.

Infectious Diseases

Human immunodeficiency virus (HIV) is a lentivirus that causes Acquired Immunodeficiency Syndrome (AIDS), a condition in humans in which there is failure of immune system, leading to life-threatening opportunistic infections.

Although HIV-1 infection is responsible for most of the global AIDS pandemic, HIV type 2 (HIV-2) is an important cause of disease in a number of regions of the world. Quantitative RT-PCR (q-PCR) is a sensitive and reliable method to detect HIV-2 and quantify viral load in human serum.

An **Echovirus** is a type of RNA virus that belongs to the genus Enterovirus of the Picornaviridae family. Clinical manifestations vary from mild to lethal and acute to chronic, and are associated with aseptic meningitis (mostly serotypes 2,5,6,7 and 9), muscle weakness, paralysis, exanthems, enanthems, pericarditis, myocarditis, common cold, conjunctivitis, infantile diarrhea, and acute febrile respiratory illnesses. The laboratory diagnosis of Echovirus and other Enteroviruses is cell culture isolation, followed by serotype identification by a neutralization assay in human serum and CSF samples by microscopy.

Conclusion:

Cambridge Biomedical specializes in immunological methods in order to diagnose and study, not limited to, but including, *neurodegenerative diseases, Hematological Malignancies, Immunomonitoring, rare diseases, Celiac's disease, diabetes mellitus, oncology, and infectious diseases.*



References:

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2. Timothy M Cox., Fabry Disease: Perspectives from 5 Years of FOS. **Chapter 9 Biomarkers in lysosomal storage diseases**

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Dr. Gupta is a Senior Research Scientist at Cambridge Biomedical Inc. She is a cell biologist and immunologist with specific expertise in developing various assays based on techniques such as flow cytometry (FACS), Luminex, ELISA, Mesoscale Discovery (MSD), immunohistochemistry, and cell-based assay techniques, among others. Dr. Gupta has extensive and broad experience in oncology and inflammatory diseases, hematological cancers, skin disorders, cancer and melanoma research, and biologics and recombinant antibody technology.

She previously worked in these fields at the Massachusetts General Hospital, Abbott Bioresearch Center, and Shire Human Genetic Therapies.

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