



Princess Lilian Professorship 2019 Pr Alain FISCHER

Professor of Experimental Medicine Collège de France – Paris

- Prix Halpern, 1984
- Prix Behring-Metchnikoff, 1992
- Prix du Comité du rayonnement français, 1994
- Prix de Médecine de la Fondation Jung (Hambourg), 1998
- Prix de la Fondation NRJ-Institut de France, 2000
- Prix de Pédiatrie Pierre Royer, 2000
- Prix Louis Jeantet (Genève), 2001
- Prix Novartis d'immunologie clinique (Stockholm), 2001
- Membre de l'EMBO, 2002
- Correspondant étranger de l'Académie royale de médecine de Belgique, 2003
- Prix A. Philipson de pédiatrie (Stockholm), 2003
- Prix René Descartes de la Communauté européenne, 2005
- Docteur honoris causa de l'Université de Zurich, 2005, Athènes 2010, Buenos Aires 2010
- Prix de Médecine moléculaire (Debrecen, Hongrie), 2007
- Grand prix Inserm, 2008
- Officier de la Légion d'honneur, 2010
- Prix Avery-Landsteiner (Glasgow), 2012
- Grand Prix Claude Bernard de la Ville de Paris, 2013
- Prix Sanofi/Pasteur, 2013
- Japan Prize, 2015

The University of Liège Medical School and GIGA Institute are extremely proud and honored to receive Pr Alain FISCHER from the prestigious Collège de France in the framework of the Princess Lilian Professorship 2019. Alain Fischer is one of the most prominent immunologists in the world and a leader in the field of hereditary immune deficiencies (HID) and their treatment by gene therapy. His major scientific achievements are summarized below.

Normal and pathological T-cell development

T lymphocytes, key players of the adaptive immunity, are essential for life after a few months. Children who are born without T cells (severe combined immune deficiencies or SCID) are not able to defend themselves against bacterial, fungal, parasitic or viral infections.

With Jean-Pierre de Villartay, Alain Fischer initiated the exploration of these pathologies in order to understand the molecular bases of T-cell development, from bone marrow to the thymus. They showed that a variety of SCID is characterized by an increased sensitivity of patients' cells to inonizing radiation, which suggested a defect in a system of DNA repair necessary for T- and B-cell differentiation.

A few years later, through the use of genetic mapping, they identified the responsible gene and showed that the defient protein *Artemis* plays an essential role in a repairing pathway of DNA breaks named repair by non-homologous junction of breaks of the two DNA chains. Indeed, during T- and B-cell differentiation, the genes coding for some receptors for antigens (TCRs and BCRs) are submitted to the process of genetic recombination that is the source of clonal diversity, each clone recognizing a specific antigen. This recombination includes a step of DNA fragmentation that is followed by a repairing that involves Artemis. More later, the same research strategy based on another variety of SCID led to the identification of another factor of non-homologous repair of DNA breaks named *Cernunnos* that is implicated in the final phase of ligation of DNA fragments.

With Geneviève de Saint-Basile, he has devoted much effort to the genetic and molecular characterization of a form of SCID whose gene is located on the X chromosome and is responsible for a complete defect in the development of T lymphocytes and Natural Killer (NK) lymphocytes. They were able to locate the gene locus on the X chromosome in 1984 and especially to observe a partial spontaneous improvement of this serious pathology related to the occurrence of a 'revertant'. This phenomenon is the consequence of a somatic mutation event occurring in a T-cell precursor which, by chance, led to the correction of the inherited mutation and thus allowed, by selection, the differentiation of T lymphocytes. This observation allowed to estimate the number of T cells that can be generated from a single precursor. It was also the key element of the reasoning that led to the development of gene therapy for this disease (see below): a single cell dividing a large number of times can generate a very large number of T lymphocytes. They have considered that a form of 'natural' gene therapy had by chance partially corrected the child's SCID. This observation illustrates how detailed analysis of an exceptional situation can contribute to understanding physiology (T-cell development dynamics) and to developing new therapeutics.

The diversification of the antibody response and its genetic anomalies

B cells produce antibodies (immunoglobulins) that recognize antigens by their so-called variable region and activate cellular functions by their constant regions. The variable regions are generated by the genetic recombination process as described above. Constant regions are of several types (M, G, A, E) with different functions. A second process of genetic recombination allows, during an immune response, to modify an immunoglobulin such that 1) the constant region changes from M to G, A, or E, and 2) the variable region acquires (after selection) a higher affinity for the antigen. These are respectively isotypic immunoglobulin (CII) switching phenomena and somatic mutations. These B-cell events occur in secondary lymphoid organs (lymph nodes, spleen) and increase the immune capacity of B cells against infectious agents.

A systematic and in-depth study of patients with HID characterized by a CII defect, particularly led by Anne Durandy, led to the identification or clarification of the role of molecules essential to this secondary diversification of the immune regions: it is successively the molecule called CD40L, present on the surface of the T lymphocytes necessary for the production of immunoglobulins (cooperation T-B), the molecule Activation Induced Deaminase (AID) which induces the modifications of the DNA, responsible for the initiation of the process of recombination and somatic variable-region mutations of Uracil N Glycosylase, which "cleans" the lesion of DNA generated by AID (and DNA mismatch repair enzymes). The molecule AID, first identified by T. Honjo in Kyoto (Nobel Prize 2018) and whose importance has been revealed by the study of human hereditary pathologies, is often called the *'mutator'* of immunoglobulin genes.

The cytotoxic function of T lymphocytes and its genetic abnormalities

One of the effector functions of T lymphocytes (and NK lymphocytes) consists of the destruction of other cells infected by microorganisms (viruses in particular) but also of tumor cells. These 'killer' lymphocytes are an essential process of anti-infectious immunity. It is known that it is linked to the excretion of the content of so-called secretory cytoplasmic granules, in contact with the target cell. These proteins (perforin and proteases) cause the death of the target cell according to the so-called apoptosis process. With Geneviève de Saint-Basile, the laboratory has been able to identify a series of hereditary diseases all characterized by a functional defect of cytotoxic capacity of T (and NK) lymphocytes. This work presents several sources of interest. They point out that a lack of cytotoxicity actually leads to an exacerbated immune response, as if the killer lymphocytes were physiologically

necessary for the termination of an immune response. They led to the identification of a series of molecules whose function is to allow the exocytosis of cytotoxic granules according to a structured program: intracellular migration, membrane binding, activation and membrane fusion. Finally, in a remarkable way, this process presents surprising molecular analogies with that of the exocytosis of the granules that contain neuromediators at the level of the synapses between nerve cells, and this despite differences in scale of time and size. Thus, a similar biological issue, the regulated excretion of proteins in contact between cells, has received a similar solution in two distinct cell types.

This work has also shown that these genetic diseases cause a syndrome called 'lymphohistiocytic activation syndrome' in which the persistent secretion of interferon γ is critical. These results show how cytotoxic cells contribute to terminating an antiviral immune response and open an interesting therapeutic path by neutralizing the effects of interferon γ .

Genetic abnormalities of the immune system and autoimmunity

Autoimmune diseases are common and their prevalence increases with age. They painfully testify that cells of the adaptive immune system (T and B lymphocytes) are able to recognize self components. These self-reactive lymphocytes are not normally pathogenic, that is, they are not, outside autoimmune diseases, involved in immune responses. How are they effectively placed under control? The answer to this question of interest, both theoretical (tolerance to the self) and medicine, comes again, in part, from the study of rare hereditary diseases responsible for susceptibility to autoimmunity. Their main contribution with Frédéric Rieux-Laucat concerns the identification of inherited gene abnormalities that encode a protein called FAS in patients with lymphoproliferative syndrome with autoimmunity. FAS is a membrane receptor present especially on the surface of T and B lymphocytes. Its binding by a ligand, the FAS ligand present on the surface of activated T cells, causes the death of chronically activated lymphocytes (which is exactly the situation encountered for lymphocytes specific for self antigens). This molecular communication system, which can act intracellularly ('cis') or intercellularly ('trans'), thus controls the possible escape of self-reactive T and B lymphocytes.

It has also been shown that somatic (acquired) mutations of the same gene can promote the emergence of selfreactive lymphocytes and thus autoimmune diseases, since such lymphocytes acquire a selective survival advantage. This observation is the basis for an hypothesis that can account for common autoimmune diseases: the accumulation of somatic mutations of genes controlling death or cell division in self-reactive T cells and/or B cells, would favor their escape and therefore the induction of autoimmunity, according to a model close to oncogenesis. This hypothesis is testable (and under test); it is likely to lead to the identification of new molecules important in the control of autoimmunity.

Today, the analysis of hereditary defects of the immune system, in particular of its adaptive component (T and B lymphocytes) through the identification of genetically determined defects, continues. Current genome sequencing capabilities, such as exomes, *ie* coding portions of genes or the entire genome, greatly facilitate this approach. Thus, many mutations are being studied in relation to defects in the development of T or B lymphocytes in the occurrence of autoimmune or inflammatory pathologies. Some of these diseases correspond to a rare genetic form (monogenic cause), or more frequent pathologies, for example Crohn's disease (inflammatory bowel disease). It is expected from these studies to be able to identify molecular pathways whose more complex anomalies could explain these pathologies and thus open up possible therapeutic leads.

They postulate that the continuation of the study of models of rare genetic diseases responsible for autoimmune diseases will allow to better understand the functioning of the different systems of physiological control of the reactive lymphocytes against the components of the self. In parallel, the systematic search for somatic alterations of the genome in reactive lymphocytes against the self components (see above) will test the hypothesis of the role of the accumulation of such mutations in the genesis of autoimmunity and to identify possible therapeutic targets.

Genetic abnormalities of the immune system and gene therapy

Based on the progress of knowledge of the pathophysiology of HID, Alain Fischer sought to develop therapeutics for these pathologies, some of which could then be applied to the treatment of other diseases. This work concerns three areas: the use of monoclonal antibodies, hematopoietic stem cell allograft and gene therapy.

On the observation that in the rare patients whose leukocytes lack leukocyte adhesion proteins ' β 2 integrin' (which results in a very serious predisposition to infections due to a lack of adhesion and migration of leukocytes), there is a lower capacity for rejection of bone marrow transplant; They proposed the injection of a *monoclonal anti-\beta2 integrin antibody* as a method of preventing rejection of bone marrow transplant and this approach has been partially successful.

The Epstein Barr virus (EBV) causes in immunocompromised individuals (genetically or because of immunosuppressive treatment after organ allograft for example), an uncontrolled proliferation of B cells, because this virus selectively infects these cells and induces their cell division. In the absence of an effective immune response, this cell proliferation is deadly. They have shown that the injection of such monoclonal antibodies specific to B cells causes these cell proliferation to stop and in many cases to heal. These results have, in a way, prefigured the current success of monoclonal antibodies in the treatment of primary lymphomas of B cells. They have also, according to the same principle, participated in the first applications of the therapeutic use of monoclonal anti-B-cell antibodies to treat an autoimmune disease (autoimmune hemolytic anemia) thus destroying B lymphocytes, including those that produce pathological autoantibodies.

HEMATOPOIETIC STEM CELL (HSC) ALLOGRAFT

The growing knowledge of the pathophysiology of many severe hereditary immune system pathologies has led to the treatment of some of these diseases by HSC allograft in order to replace functional cell-deficient cells.

Based on this simple principle, tangible results have been achieved for a number of HID, inflammatory and autoimmune diseases (mevalonate kinase deficiency, *FOXP3* deficiency) and metabolic diseases (adrenoleukodystrophy).

GENE THERAPY OF HEREDITARY IMMUNE DEFICIENCIES

Despite these successes, HSC allogeneic transplantation has limitations inherent in the risk of fatal complications that may be too great when the donor and recipient do not share sufficient HLA tissue group compatibility. An alternative is gene therapy. The principle of this is based on the introduction of a normal copy of the mutated gene into the stem cells of the hematopoietic system so that the daughter cells carry this 'therapeutic' gene and are able to express the deficient protein in the patient. The stem cells are modified *ex-vivo* with the aid of a viral vector (retrovirus) whose characteristics allow the integration of its genome into the cellular genome. Thus the therapeutic gene is replicated during each cell division and, through a system for regulating its expression (transcription), the corresponding protein produced.

The first application developed with Marina Cavazzana-Calvo and Salima Hacein-Bey-Abina was the treatment of X-linked SCID, based on the observation of the selective advantage conferred on the 'revertant' precursors of T lymphocytes. The approach proved to be fruitful as, for the first time, it was possible to correct, in a stable manner over time, the manifestations of the disease. Ten patients in Paris, then 10 in London were treated, 17 of them live today in good conditions with a functional immune system, with a median decline that exceeds 10 years.

Nevertheless, this success was tempered by the occurrence of cases of leukemia (5 cases/20) consecutive to the aberrant deregulation of oncogenes located near the site of integration of the retrovirus. Although these leukemias were cured in 4 out of 5 cases, their occurrence led to a cessation of these trials, a break put to advantage to understand the mechanism of this oncogenic effect. It is now identified; it is linked to the ability of a regulatory element of the viral vector to permanently transactivate a gene located close to the site where the virus was integrated. If this gene is an oncogene, its aberrant expression accounts for the risk of leukemia. This work has led to an effort of wide international collaboration in the development of modified vectors in which the transactivator element has been removed. Such vectors have been shown to be experimentally effective and are currently being tested in new clinical trials treating several diseases: adrenoleukodystrophy, SCID, Wiskott-Aldrich syndrome. The next few years will establish whether, in fact, their use can maintain the effectiveness demonstrated while reducing the risk of side effects. The results are encouraging so far, but caution is needed.

An unexpected aspect of this research concerns the analysis of human T cell population dynamics. In treated patients, all T cells carry a molecular signature corresponding to the integration at a specific location of the viral vector cell genome. This signature can be identified and the number of cells bearing the same signature, therefore descendants of the same progenitor cell, can be quantified. It is therefore possible to follow the number of progenitors involved in the differentiation of lymphocytes, the quantitative variations of this diversity, and the abundance of each clone. By coupling this information with the study of the clonal diversity of newly formed T lymphocytes/TCR and the 'memory' T cells (previously involved in an immune response), it becomes possible to generate a dynamic image of the evolution of lymphocyte T populations *in vivo*, in particular with regard to their longevity, their renewal and their expansion during an immune response.

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