EDITORIAL

Predictive immunomonitoring — The COST ENTIRE initiative

1. Background

The main role of the immune system is to protect an individual from threats coming from the environment (i.e. infections), as well as those coming from the individual itself (i.e. tumors and autoimmunity). However, due to its enormous variability and plasticity, the immune system may also exhibit deleterious effects under numerous circumstances.

Inflammation is a phenomenon accompanying both beneficial and detrimental immune responses. Induction of inflammation is a complex process that helps the immune system to eliminate threats and restore homeostasis in the body. However, chronic inflammation often leads to damage of the cells and tissues leading to a group of diseases known as IMIDs. IMIDs constitute a major medical and social problem globally where up to 10% of the population suffers from these diseases [1]. Understanding the pathogenesis of these diseases has allowed therapeutic targeting of molecules that are critical in the initiation/maintenance of inflammation or in immunosuppression.

After the initial success of using tumor necrosis factor (TNF) antagonists in the treatment of a patient with rheumatoid arthritis, treatment attempts have been performed in other IMIDs by blocking TNF [2], other pro-inflammatory mediators (i.e. IL-1, IL-6) [3,4], or by targeting molecules that have important functions in immune responses such as immune cell trafficking [5]. In addition, application of cytokines for the treatment of certain diseases, or using antibodies that lead to the depletion of certain cell subpopulations has been successful [6,7]. The application of biological therapies radically changed the natural disease course of many IMIDs and improved quality of life of patients and their families. Despite the major clinical efficacy of many biologicals, however, these drugs are still not a universal solution for all IMID patients. Indeed, biologicals were not beneficial for all patients suffering from a particular disease. For example, TNF antagonists have beneficial effects in 60% of the RA patients, but only achieve low disease activity in 30%. In some cases biological drugs turned out to have adverse effects by deregulating immune responses [8]. These drugs are contraindicated in certain patients and can increase the risk for serious infections and/or malignancies. Furthermore, another drawback is that biological therapies may be up to one order of magnitude more expensive than classical therapies, having a substantial impact on societal health funding [8]. These findings have led doctors, scientists, health authorities and the pharmaceutical industry to re-evaluate the application of biologicals, imposing the need for a careful stratification of patients to ensure a maximum cost-benefit approach.

This is a demanding task because: 1) the immune system of each individual is shaped in a unique manner by interactions between the individual genetic background and external factors. These immune determinants specific for the individual could be called "immunotype" (by analogy with the notions genotype and phenotype) and remain to be defined with a set of reproducible assays; 2) resetting of the diagnostic and grading criteria with inclusion of immunological parameters related to the pathogenesis of IMIDs became an urgent need as IMIDs are diseases with deregulated immune responses; and 3) despite unprecedented growth of immunological knowledge in the last decades, including insights from many different animal models that are crucial for the understanding of the pathogenesis of many diseases, the transfer of this knowledge to human practice remains scarce. Direct translation of results obtained from experiments in laboratory animals to the human system suffers from serious limitations [9–11]: for instance, most of the laboratory animals are inbred and maintained in controlled housing conditions leading to poor reproducibility in other similar strains of animals or after environmental changes. Furthermore, for a number of immune parameters involved in the pathogenesis of IMIDs, there is neither standardized testing nor reference values in pre-clinical laboratories. In addition, immunological parameters vary within broad ranges in the human population, which are also partially due to the distinct genetic makeup of individuals.

Many of the IMIDs are still defined by a set of clinical findings, but do not include immunological parameters that are important for the pathogenesis of the diseases. Defining the immune signatures of a specific disease is hampered by the fact that many of these diseases are relatively rare, making it difficult to collect a sufficient number of clinical samples to be analyzed using the same methodology, reagents

and platforms in order to get comparable results. Yet, it would be necessary to find underlying immune signatures for: 1) diagnosis (to classify disorders according to their immunobiology, for example ANCA-associated vasculitis), 2) prognosis (for example association of anti-SSA antibodies with congenital heart-block), 3) prediction of treatment response (such as whether anti-CCP predict better response to rituximab in RA), and 4) definition of immunological rather than clinical remission.

In summary, rational immunological criteria for diagnosis, prognosis, treatment and remission of IMID are still missing, while the options as well as the costs of biological therapies are ever increasing.

2. Aim of the COST-ENTIRE initiative

To address these issues, European centers within the Federation of Clinical Immunology Societies (FOCIS, an organization that has enrolled 54 centers of excellence representing about forty thousand physicians and scientists) have established a European network to actively work to overcome the aforementioned problems. The established network is dedicated to improve health through an interdisciplinary approach in the field of clinical immunology. The partnership is built through the COST Action BM0907 ENTIRE, European Network for Translational Immunology Research and Education: "From immunomonitoring to personalized immunotherapy" (www.entire-net.eu). The main objective of this action is to define and characterize the immunotype of healthy individuals and patients with IMIDs before, during, and after targeted immunomodulation. To address the basic goal of the ENTIRE, participants will employ an integrative approach relying on state-of-the-art technology in the field of single nucleotide polymorphism detection, mRNA expression studies, proteomics, liquid array based assays and flow cytometry. Exchange of samples and harmonization of standard laboratory protocols between centers of the action will enable participants to expand the groups of healthy individuals and patients. Analysis of samples from different European countries will account for the genetic variability of the European population. This progress will result in a shift from predominantly opinion-based clinical decision making to evidence-based decision making.

The first steps in implementing the principles of evidence-based medicine within the ENTIRE action were made by defining the level of evidence of specific in vitro diagnostic tests, used in clinical laboratories, for the prediction of adverse reactions to biologicals. Evaluation of potential tests was performed using standardized searches of public databases Medline, EMBASE and Cochrane with consensually defined key words. The consensus was accomplished by representatives of the six participant centers and with the help of a professional librarian. Search results and conclusions were recorded in the form of Critically Appraised Topics (CATs). Review of the CATs led to the undivided opinion of the participants that there is very little evidence for the existence of an accepted common knowledge regarding laboratory testing.

During the FOCIS annual meeting in 2009, the need for immunophenotyping standardization of higher order polychromatic panels was discussed and a Human Immunophenotyping Consortium (HIP-C) stemmed from the FOCIS Network of Consortia to address differences in sample handling, flow cytometry panels, instrument set-up, and data analysis that all contribute to impede standardization of flow cytometry in clinical trials. HIP-C has briefly reviewed the above mentioned areas and proposed a model to minimize variation in multi center studies [12]. In brief, this model appoints a central laboratory "harmonizing" remote sites, by providing for example a standard operating procedure (SOP) and centralized training. In such a scenario samples would still be collected, processed and acquired at local sites, but using procedures that have been standardized and certified by a central laboratory. Several groups from the ENTIRE network are actively supporting HIP-C by testing the panels agreed upon at the Immunophenotyping Technical Meeting at NIH in February 2011, in a first exercise to standardize flow cytometry staining and acquisition [13]. Results and conclusions from this effort are expected to be relevant for our understanding of current shortcomings in the standardization of multicolor immunophenotyping in diagnostic settings and ways to overcome these obstacles in order to employ multicolor flow cytometry in clinical trials and diagnostics.

Another important goal of the ENTIRE network is to define a panel of whole blood assays, along with standardization and production of a SOP for each particular test. Evaluation of this panel will be carried out for immunological drugs, including drugs known to have caused adverse events.

To facilitate communication between the ENTIRE participants, but also as a service to the general scientific community, we have created the ENTIRE action web site (www.entire-net.eu). Here we publish our collection of CATs, guidelines and adopted SOPs, to make them available for interested parties. Immunological scientists as well as experts in related topics are welcome to submit their comments, unpublished observations and results to enrich the content of www.entire-net.eu. The website will serve as a platform to both fulfill scientific objectives, serve for education, and provide a rapid way to spread our findings. As a result, we hope that our efforts will help to shift the decision process in the field of clinical immunology from educated guessing to a more evidence-based procedure.

Direct benefits from defining immunotypes in health, their changes in disease and during therapy will be helpful for doctors when evaluating patients and making therapeutic decisions. It will enable the personalization of treatment algorithms from introduction of the drugs, through determination of the maintenance dose, to the tapering and ending of therapy. Subsequently, patients will be more effectively treated with fewer side effects and the quality of life for both them and their families will improve. Society will have a lower overall morbidity and higher productivity. Evidence based immunotypes and immune signatures of IMIDs will be available also for the pharmaceutical industry. This would facilitate the identification of therapeutic targets and the development of drugs that act on them. In addition, the pharmaceutical industry and regulatory bodies will have guidelines for the parameters of the immune response in testing biological (and other) drugs. We hope that this will facilitate the process of licensing new drugs and reduce the possibility of repeating adverse scenarios as in the past [9–11]. An indirect benefit of the ENTIRE network activities is

the establishment of strong links between European centers dedicated to clinical immunology research and to the overall development of the European Research Area.

Conflict of interest

The author(s) declare that there are no conflicts of interest.

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