Comparative Immunology, Microbiology and Infectious Diseases 59 (2018) 57–62

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Immune response biomarkers in human and veterinary research

Alba Llibre[a,b], Darragh Duffy[a,b,*]

[a] Immunobiology of Dendritic Cells, Institut Pasteur, Paris, France
[b] INSERM U1223, Paris, France

ARTICLE INFO

Keywords:
Biomarkers
Cytokine
Chemokine
Type I interferon
Tuberculosis

ABSTRACT

Biomarkers are increasingly utilised in biological research and clinical practice for diagnosis of disease, monitoring of therapeutic prognosis, or as end points in clinical studies. Cytokines are small molecules that orchestrate immune responses and as such have great potential as biomarkers for both human and veterinary fields. Given the ease of sampling in the blood, and their high prevalence in clinical applications we will focus on protein detection as an area for biomarker discovery. This is facilitated by new technological developments such as digital ELISA that have led to significant increases in sensitivity. Two highly relevant examples include type I interferons, namely IFNα, that is now directly quantifiable by digital ELISA from biological samples. The application of this approach to the study of the unique bat interferon response may reveal novel findings with applications in both human and veterinary research. As a second example we will describe the use of CXCL10 as a disease biomarker in Tuberculosis, highlighting findings from human and mouse studies that should be considered in veterinary research. In summary, we describe how cytokines may be applied as novel biomarkers and illustrate two key examples where human and veterinary research may complement each other in line with the One Health objectives.

1. Introduction – the clinical potential of protein based biomarkers

Biomarkers are a broad category of medical indicators that can be measured objectively to determine different medical states or responses to an intervention. Therefore, they can be used for basic diagnosis, therapeutic prognosis, or as end points in clinical studies. Examining how biomarkers are used in research and the clinic in human and veterinary fields can be informative for both fields. Biomarkers may consist of nucleic acids, proteins, metabolites, and even images as long as they can be recorded and measured consistently and objectively. With the huge progress in genomics since the successful sequencing of the human genome, much attention has focused on the potential use of genetic biomarkers. However, despite dramatic reductions in sequencing costs and increases in capacity, the clinical potential of this approach still remains largely unmet with only 55 unique pharmacogenomic biomarkers (many of these biomarkers have multiple indications so the overall number is higher) currently approved by the Food and Drug Administration (FDA) for human treatment decisions [1]. The majority of these genetic markers are single nucleotide polymorphisms (SNPs), although more recently Next Generation Sequencing (NGS) techniques are being successfully applied in particular for cancer mutation detection.

In contrast to the use of genetic markers, an estimated 325 proteins can be reliably measured in human plasma by conventional clinical chemistry or Enzyme-Linked ImmunoSorbent Assay (ELISA), with concentrations ranging between 10^{-3}M and 10^{-12}M [2]. Of these proteins an estimated 175 are currently approved by the FDA as clinical biomarkers highlighting the advanced maturity of these technologies and also potentially their greater clinical relevance as compared to genetic biomarkers [3]. In addition, there is an estimated 4000 secreted proteins that are present in the circulation at concentrations less than 10^{-12}M, offering huge potential for new biomarker discoveries. Novel technologies such as digital ELISA and Mass Spectometry based approaches now have the ability to unlock this potential for both human and veterinary research.

2. Cytokines and Chemokines as immune response biomarkers

Blood and plasma-borne biomarkers are not only desirable for their accessibility, but also because they offer the potential for serial monitoring. Unlike SNPs which are static, protein responses are dynamic and can therefore potentially reflect disease status or even responses to therapeutic intervention. Plasma carries information from most organs

* Corresponding author at: Immunobiology of Dendritic Cells, Institut Pasteur, Paris, France.
E-mail address: darragh.duffy@pasteur.fr (D. Duffy).

https://doi.org/10.1016/j.cimid.2018.09.008
Received 19 December 2017; Received in revised form 22 May 2018; Accepted 17 September 2018

and tissues [3,4], therefore, by analysing plasma protein composition, we can potentially capture the variation occurring in a wide range of human and animal tissues due to different disease states. There is a large dynamic concentration range for plasma proteins [4], allowing their classification into three main categories: high abundance plasma proteins such as albumin (35–50 mg/ml), immunoglobulin proteins (5–18 mg/ml) and tissue origin proteins or cytokines, with the latter being at the lower concentration (0–5 pg/ml).

Of particular interest for immunological studies are cytokines, which are small signalling molecules that orchestrate immune responses by enabling cell to cell communication and recruitment of immune cells to infection sites [5]. Cytokines can be further subdivided into interleukins (IL), interferons (IFN), chemokines and tumor necrosis factors (TNF), which may promote either pro-inflammatory or anti-inflammatory responses. They have the capacity to stimulate and modulate the immune system and are therefore great indicators of normal immunological processes, pathological processes or responses to treatment. Thus, cytokines have become highly relevant immune response biomarkers, and they are currently being used in a wide range of clinical situations. Relevant examples include the potential diagnostic value of IL-17 for both rheumatoid and psoriatic arthritis (24.3 ± 9.6 pg/ml in the inflammatory joint) [6,7]; elevated levels of IL-1β, IL-6 and TNFα that are associated with severity and progression of chronic liver disease (31.4 ± 3.0 pg/ml, 23.0 ± 4.0 pg/ml and 21.5 ± 3.9 pg/ml, respectively, in cirrhotic liver) [8]; and a cytokine signature based on IL-18, TNFα and IL-4 has been proposed as a predictive biomarker for Glatiramer acetate responsiveness for the treatment of multiple sclerosis [9]. These examples illustrate how cytokines have become invaluable biomarkers for diagnosing disease, for measuring progression/severity of disease states and predicting and monitoring the effects of therapeutic interventions. This is not only true for human immunology, but numerous examples can also be found in veterinary medicine. A good illustration of this is the study carried out by Bertuglia and colleagues, in which the kinetics of pro-inflammatory cytokines such as TNFα were associated with equine osteoarthritis (mean of ~100 pg/ml in synovial fluid and ~35 pg/ml in serum) [10]. Furthermore, changes in IFNα, IL-8 and TNFα have been described during classical swine fever infection (for example, peak between 3 and 8 days post-infection of IFNα reaching levels up to 1100 pg/ml) [11] and particular cytokine profiles have been associated with natural African trypanosome infections in cattle (mean of ~280 pg/ml of IL-10 in infected cattle *versus* ~150 pg/ml in uninfected animals, as well as ~5 pg/ml of TNFα and ~2.8 pg/ml in infected and uninfected animals, respectively) [12]. Overall, cytokine biomarkers hold great potential to monitor health and disease states both in the field of human and animal medicine.

3. Type I interferons – key immune response cytokines

The very first cytokine identified was the description of a soluble factor that protected cells from viral infection made by Isaacs and Lindenmann in 1957 [13,14], thus initiating this field of research. Despite this discovery now more than 60 years ago, the direct measurement of type I IFN protein in biological samples has remained challenging. Type I IFN mRNA is usually present at only trace levels in peripheral blood mononuclear cells (PBMCs) from healthy individuals, and current ELISAs have proved either insensitive or unreliable for detection of the protein leading to the development of a wide variety of proxy assays of type I IFN signalling [15–19]. Such low levels of circulating IFN protein likely reflect the high biological potency of type I IFN, with most cell types expressing a type I IFN receptor (IFNAR). Indeed, in addition to their role in anti-viral protection, it has become clear that inappropriate or excessive exposure to IFN can have major detrimental effects [20], so that a balance must be struck between the ability to fight infection *versus* the risk of inflammatory disease. This latter point is well illustrated by the very strong correlation of enhanced type I IFN signalling in systemic lupus erythematosus (SLE), juvenile-onset dermatomyositis (JDM) and type I interferons [21]. Furthermore, in humans multiple species of type I IFN exist, with this heterogeneity arising from distinct encoding genes, including 13 functional α genes and one β gene situated syntenically on human chromosome 9p. Differences in IFN-subtype production, activity site, and profile allows for a subtlety in immune function that is presently poorly understood.

Despite its key role in many diseases, the study of type I IFN protein levels in biological samples has been challenging until recently. Conventional sandwich ELISA is the most widely used method for detection of IFNα protein. However, despite being specific, simple and rapid, type I IFN ELISA assays present important limitations, in particular insufficient sensitivity. In addition, the measurement of all IFNα subtypes requires the use of multiple assays each with their own detection capacity and sensitivity. The limit of detection (LOD) of classical ELISA is within the picomolar range, which is insufficient to detect IFNα protein in biological samples. As previously mentioned, this fact possibly reflects the biological potency of type I IFN, thereby highlighting the lack of tools to monitor levels of these cytokines in infection and autoimmunity. To overcome this limitation, several biological proxy assays have been developed to quantify type I IFN by measuring induced gene expression or functional activity [15–19,22,23]. Nonetheless, it is important to emphasize that these assays do not provide a direct measurement of the IFNα protein.

However, the recent development of digital ELISA, in particular Single molecule immunoassay (Simoa) [24], represents a significant advance over previous approaches and has now made possible the direct quantification of cytokines such as type I IFNs [25]. Simoa utilizes paramagnetic beads, coupled to specific antibodies, that are isolated in femtolitre-sized nanowells specifically designed to isolate a single bead in a high throughput manner. This innovative step reduces the non-specific background fluorescent signal allowing the specific signal to be measured in a digital fashion. The results are both significant increases in sensitivity and increased confidence in low measurements as the previous non-specific technical noise has been greatly reduced. The application of this approach to the quantification of IFNα protein at attomolar concentrations has allowed the description of this protein in human plasma, cerebrospinal fluid (CSF), and cellular lysates for the first time in diverse disease cohorts ranging from autoimmunity to viral infection (median 1.6 fg/ml, interquartile range [IQR] 0.95–4.6 fg/ml in plasma from healthy individuals, median 20 fg/ml, IQR 0.60–234 fg/ml in plasma from SLE patients and median 56 fg/ml, IQR 14–120 fg/ml in plasma from JDM patients. IFNα protein could also be detected in CSF from patients with central nervous system infection; median 4,174.2 fg/ml IQR 2,437.4-11,173 fg/ml) [25]. Therefore, this novel approach may also now be applied to veterinary research questions if specific antibodies are available for the particular species of interest.

4. The unique IFNα response of bats

An area of research where this approach could provide novel insight for both human and animal fields is the immune response of bats to viral infection. Bats are recognised as a potential reservoir for many viruses such as coronaviruses, filoviruses, lyssaviruses, and henipaviruses that can cause severe disease in humans but not in bat species [26]. Likely as a result of their close co-evolutionary history with viruses, which enables them to live in a symbiotic relationship, bats are considered an important source of future viral pandemic outbreaks. To better understand this unique scenario, a recent fascinating study sequenced the IFNα locus of the Australian black flying fox, *Pteropus Alecto* [27]. This study identified in bats a highly contracted type I IFN family consisting of only 10 IFNs that includes three functional IFNα loci. A comparison of the sizes of the IFNα locus in different animals revealed that it ranged from 25 kb in fish to 1 Mb in pig, with a trend toward increasing size through evolution, with the notable exceptions of smaller sizes in chickens and bats (30 kb and 250 kb respectively)