Systemic Adverse Events with Biomedicines

Giuseppe Tridente, MD, PhD

Abstract— The core of biological agents recently introduced in human therapy includes Monoclonal Antibodies, Fusion proteins and a number of Cytokines employed in monotherapy or in association with conventional drugs. Their extraordinary impact in oncologic, autoimmune, and inflammatory diseases is impressive. However, they are usually accompanied by a complex profile of Drug-Related Adverse Events (DRAEs) with peculiar characteristics, mostly related to the proteic nature of these new agents often targeting crucial components and receptors of the immune system. Such interaction ultimately induces local and systemic reactions generated by activating or inhibiting signals, leading to cell hyperactivation and destruction, immunosuppression, eventually followed by immune rebounds after therapy discontinuation. Among the clinical consequences of DRAEs, a number of systemic syndromes are of particular concern for their rapid insurgence and severity, although expressed in a limited number of patients. We consider here eight different clinical expressions, namely the Capillary Leak Syndrome (CLS), the Cytokine Release Syndrome (CRS), the Infusion Reaction Syndrome (IRS), the Tumor Lysis Syndrome (TLS), the Systemic Inflammatory Response Syndrome (SIRS), the Macrophage Activation Syndrome (MAS), the Immune Reconstitution Inflammatory Syndrome (IRIS), and the Immune-related Adverse Events Syndrome (IrAES) not only for their clinical relevance, but also as paradigmatic examples of the mechanisms of action (MOAs) involved during biomedicine’s therapeutic administrations.

From the analysis and confrontation of both clinical features and the twine of interacting MOAs in each syndrome, it emerges a common pathogenetic background consisting in a dynamic imbalance of the cytokines network, where pro-inflammatory molecules prevail, thus generating different systemic expressions based on a common pivotal event here defined as Cytokine Dysregulation Status/Syndrome (CDS). Such pathogenetic interpretation if offered with the aim of stimulating further investigation and debate.

Introduction

The amount of biological agents introduced in human therapy in the last two decades is impressive and expanding. Those targeting or involving the immune reactivity are mainly employed in oncology, in autoimmune and inflammatory diseases, and in the control of graft rejection. More recently, an additional number of structurally similar products are being experienced in different areas of human pathology, such as infectious diseases, allergic asthma, and ocular degenerative disorders. The core of these biological agents, or Biomedicines, is represented by Monoclonal antibodies, Fusion proteins, and by a number of Cytokines exploiting relevant beneficial effects, either in monotherapy or in association with other drugs.

However, the therapeutic effects are usually accompanied by a complex profile of drug-related adverse events (DRAEs), in part similar to non-biological drugs, but with additional and relevant new features. In fact, biomedicines show peculiar characteristics with respect to conventional drugs. In particular, they have a proteic structure, and often are targeting crucial structural of the immune system.

The proteic structure of these agents is usually highly immunogenic, although recently mitigated by sophisticated procedures of “humanization” considerably lowering their “foreignness”, yet unable to totally avoid the immune reactivity of the host, and related adverse consequences. Moreover, the peculiar targeting of some biomedicines, either against immune cells and/or interfering with receptors or their ligands involved in major immune functions, ultimately induce either immunosuppressive effects, often required for their therapeutic action, or less frequently an immuno-bursting usually causing undesirable hyper-immune reactivity.

Taken together, the safety profile of these new medicines appears to be complex and dependent from different mechanisms of action (MOAs), although often moderate in their expressions with respect to other drug classes, such as conventional chemotherapeutics and non-biologic immunosuppressive agents.

An analysis on this matter has been recently published [1], and specific consequences of immunogenicity in relation to the induction and expression of DRAEs have been recently reviewed, also in this Journal [2,3].

Here, we consider the insurgence of a number of Systemic Syndromes experienced in association with the administration of monoclonal antibodies, fusion proteins, and cytokines, including some growth factors. Although most of these events are not exclusive of biomedicines, they have attracted new attention, due to the extraordinary expansion of this class of drugs, and to some peculiarities, which have better illustrated their MOA(s), and consequent strategies for their prevention and therapeutic control.

Submission date: 24/05/2014; Accepted: 25/05/2014

(*This article is partially based on Tridente G (2014) Systemic Syndromes with Biomedicines. In: Tridente G. Adverse Events with Biomedicines. Springer-Verlag Italia, pp 25-47. Reproduced with permission of Springer

Professor Emeritus of Immunology at the School of Medicine, University of Verona, Italy.

*Correspondence giustrid@gmail.com

93
Systemic syndromes are also of particular interest because of their clinical relevance, although limited to a minority of subjects treated with biological agents, as well as for their particularly complex pathogenesis. Moreover, some of these therapeutic interventions may unveil and/or enhance underlying low/asymptomatic conditions, thus complicating the clinical scenario, and the entire expression of adverse events (AEs).

Such mechanisms are not exclusive of these syndromes, being also responsible for more localized disorders, but they find the most relevant expression in the systemic outcomes, because of the serious involvement of patient’s condition, the frequent aggravation of the underlying disease(s), and the consequent imbalance of the risk/benefit ratio during treatment.

Therefore, the clinical situations described as Capillary Leak Syndrome (CLS), Cytokine Release Syndrome (CRS), Infusion Reaction Syndrome (IRS), Tumor Lysis Syndrome (TLS), Systemic Inflammatory Response Syndrome (SIRS), Macrophage Activation Syndrome (MAS), Immune Reconstitution Inflammatory Syndrome (IRIS), and the most recent Immune-related/mediated Adverse Event Syndrome (IrAES/ImAES), will be briefly considered as the most relevant disorders observable during biomedicine’s therapeutic administration, representing also paradigmatic conditions which are at the highest risk for treated patients, but which also may improve the understanding of the whole safety profile of this peculiar drug class.

Finally, an attempt to provide a comprehensive etiopathogenetic vision is offered.

Pathogenetic mechanisms

As previously mentioned, AEs to biological agents are usually related to their specific MOAs to the respective target(s) involved, and/or to their immunogenicity as proteic structures. In the first case, DRAEs are more strictly linked to the therapeutic action of the biomedicine, while in the last case they are usually not related to their pharmacological action, although they can interfere with, and appear to be more strictly dependent on the immune reactivity of the host.

DRAEs induced by specific MOAs include those related to destruction/functional blocking of the target, either represented by a cell surface receptor or part of it, and events consequent to the triggering of bound targets. In the former case, cell destruction may exert direct toxic effects or indirect consequences determined by an abrupt release of cell soluble effectors, such as cytokines. In the latter case, the binding can cause an enhancement of underlying cell function(s), leading to their overexpression, or to inhibition of specific signals, leading to immunosuppression. However, in a minor number of cases the initial drug-target binding phase may lead to overactivation of intracellular pathways able to induce violent and relevant undesirable reactions as well, before exerting full inhibitory/destructive effects on the target.

DRAEs related to the peculiar immunogenicity of biomedicines [2] can be observed as a consequence of drug loss of response (LOR), impacting the therapeutic efficiency of the agent in use, up to total clinical unresponsiveness, or as hypersensitivity reactions (HR). LOR is mostly sustained by the formation of anti-drug antibodies (ADA), mainly of the IgG class, while HR can be supported by any of the four types identified by Gell and Coombs.

Quite recently, an additional and intriguing class of DRAE related to the induction of immune-mediated events has been observed as a consequence of inhibition of specific downregulatory immune signals. This is the case of Ipilimumab, inhibiting CTLA-4 signals, thus causing a sort of “breaks off” effect on the immune system, leading to an enhanced auto-aggressive activity of immune effector mechanisms triggering a number of multi-organ serious and fatal inflammatory processes.

As expected, these mechanistic alternatives may be also concomitantly involved in specific clinical situations, thus increasing the complexity and severity of DRAEs expression. A paradigmatic situation is represented by IRS, during which any of these events can be superimposed or subsequently expressed in the same patient.

All formerly described DRAEs can be considered a consequence of drug(s) administration. However, a relevant and new additional class of events, mainly observed with biomedicines, is related to drug discontinuation. In this case, the imbalance of cell functions caused by the previous therapeutic administration is followed by an abrupt rebound, often over levels of physiological functional/numerical recovery. Such situations may lead to an aspecific inflammatory rebound, or to an immuno-inflammatory rebound directed to specific microbial antigens already present in the host.

Therefore, DRAEs with biomedicines can produce a complex variety of direct and indirect effects, related to non-immune and immune reactivity, either as a consequence of therapy, or after its discontinuation, being the major effects related to direct toxicities, cytokine releases, cell function activation/suppression, drug inefficiency, and HR (Figure 1).

At the clinical level, such reactions are usually observed as localized mild/moderate expressions, but in a minor number of cases they can be more complex/generalized, reaching high-grade severity, up to fatal consequences, as best observed in the mentioned Systemic Syndromes. During their evolution, DRAEs may show a sequence of events determined by an initial overstimulation, followed by target destruction. In the case of an immune target, such as neoplastic T lymphocytes, a biphasic wave of adverse disorders can follow, as a consequence of an immune overstimulation, followed by a rapid cell destruction leading to a massive release of intracellular products, which may trigger both toxic and stimulatory events of relevance.

The variability of clinical expression, symptomatic complexity and severity, concerns specific agents, their administration typology, patient’s sensitivity, underlying diseases, preceding and concomitant therapies, as well as a number of unknown factors. Nonetheless, their knowledge is crucial, since most of these DRAEs can be prevented, mitigated or controlled by different prophylactic and therapeutic procedures, when promptly identified.

Among biomedicines, a number of monoclonal antibodies, fusion proteins and cytokines have proved to elicit systemic effects inducing different clinical expressions, as
reported in Figure 1. In fact, a pivotal role in systemic DRAEs induction is played by inflammatory (IL-1, TNF-α IFN-γ), and pro-inflammatory (**) cytokines (IL-6, IL-7, IL-9, IL-12, IL-15-18, IL-20, IL-23, IL-27, etc.) which can be overproduced by specific cell components (T lymphocytes, monocytes, macrophages, endo/epithelial cells, etc.), but more importantly they can be promptly released in circulation and in interstitial fluids, causing or enhancing violent immune-inflammatory “storms”. Other regulatory cytokines (IL-2), some chemokines with inflammatory activity (IL-8, now CXCL8), VEGF, myelopoietic stimulatory factors (CSFs), and their therapeutic derivatives may as well contribute to the clinical manifestations. Noteworthy, some anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGFβ, IL-1ra) are also freed during the acute phase, which ultimately involves the whole compartment of cell factors contained/synthesized by targeted cells [4,5].

We consider the imbalance of the cytokine network the crucial pathogenetic event underlying the insurgence and evolution of Systemic Syndromes induced by Biomedicines.

(**) The pro-inflammatory definition should indicate factors promoting inflammatory effects by inducing proliferation and/or synthesis of inflammatory agents, but often is comprehensive of the inflammatory cytokines

**Capillary Leak Syndrome**

First clinically described in 1960 as acute episodes of collapse due to a massive albeit reversible plasma extravasation causing shock and diffuse edema, initially identified as Clarkson disease, the syndrome was later related to cytokine release leading to an increase in vascular permeability, and subsequently defined as Capillary Leak Syndrome (CLS) or as Vascular Leak Syndrome (VLS) [6,7].

Later on, different incoming stages of the Acute Disorder were better identified [8], as:
- **Prodromic phase**, characterized by a flu-like syndrome, malaise, body weight gain, fatigue, muscular weakness and myalgia, eventually followed by pyrexia, abdominal pain, diarrhea and vomiting;
- **Leak Phase**, during which up to 70% of plasma can flow out of the vascular system causing acute hypotension, edema of face, trunk and extremities, hemococoncentration, hypoalbuminemia, oliguria, thirst and cool skin. At this stage, more serious complications may occur, such as signs of localized ischemia, renal failure, stroke, deep vein thrombosis, and rhabdomyolysis. Recently, a serious acute myocardial involvement, leading to cardiogenic shock, has been also reported [9].
- **Post-Leak Phase**, when symptoms and signs revert rapidly while fluids are recruited back into circulation; diuresis promptly increases, but the massive fluid rebound induces diffuse visceral edema, among which pulmonary edema and cardiopulmonary failure are the most serious consequences. The high volume of fluids usually introduced in these patients during the leak phase, in order to counteract the extravascular fluids loss, also influences such complications.

**Chronic CLS** is a very rare and questionable clinical state characterized by a non-cyclic peripheral edema associated with hypoalbuminemia, but not with hypotensive acute episodes.

**Local CLS**, or brain-capillary leak syndrome also defined as **Reversible Posterior Leukoencephalopathy Syndrome** (RPLS), is considered a peculiar localization of CLS, associated with hypertension, fluid retention determining cerebral edema, headache, visual loss and seizures, following cytotoxic damage of the endothelial capillary brain compartment [10]. However, CNS can be also involved during systemic CLS, with life-threatening consequences [11].

The pathogenesis of CLS is mainly based on a primary damage at the endothelial capillary level, greatly increasing vascular permeability, although the precise mechanistic events are substantially unknown. Sequential multifactorial events have been proposed, such as: (a) an initial primary toxic effect on endothelial cells; (b) activation of endothelial cells and leukocytes, causing (c) additional excretion of pro-inflammatory cytokines and other phlogistic products, leading to (d) an increased damage of endothelial permeability.

CLS has been observed in various human pathologies, such as sepsis, trauma, burns, pancreatitis, and in lymphoproliferative disorders, including lymphoma and monoclonal gammopathy. Moreover, fatal CLS has also been observed in association with C1 inhibitor deficiency during an acute exacerbation of a dermatological disorder [12].

The syndrome may follow various therapeutic procedures, such as stem cell transplantation, and a number of non-biological, anti-neoplastic, immunosuppressive (Cyclosporine, Cyclophosphamide, Mitomycin C, Cytosine arabinoside, Gemcitabine, Docetaxel, Paclitaxel), and dermatological (Acitretin) treatments [13]. However, the introduction of biomedicines in oncologic, rheumatologic, and dermatologic disorders has focused new attention on CLS insurgence and pathogenesis. In particular, additional information has evidenced (e) the role of endothelial cell retraction with released cell interconnections, (f) the association of IgG monoclonal gammopathy with CLS manifestation not related to therapy, (g) the crucial role of endothelial apoptosis, through a more direct evaluation of the overall role of cytokines, monoclonal antibodies, and inflammatory mediators at endothelial level, both in vivo and in vitro [4,8,14].

Among cytokines, some Interleukins (IL-2, IL-3, IL-6, TNFα), together with therapeutic analogues as Aldesleukin (IL-2), and Interferons (IFN-α, IFN-β1b) have been identified as relevant CLS inducers. Other peculiar cytokines such as chemokines with inflammatory activity (CXCL8), growth factors (GM-CSF, G-CSF) and their therapeutic derivatives (Oprelvekin, and Filgrastim, Pegfilgrastim, Sargramostim –or Grastims-), may induce CLS even at low doses. Some immunotoxins, i.e. specific mAbs or fusion proteins conjugated with microbial toxic agents, such as Denileukin-diftitox (DT), could also show the same capacity related to neoplastic T cell targets (T cell lymphoma, Mycosis fungoides, Sézary syndrome) acutely disrupted by the toxic
agent, leading to abrupt release of their intracellular cytokines [1,15-17]. Some of these agents can induce fatal CLS, in particular Muromonab, Aldesleukin and Denileukin-DT, for which a Black Box Warning (BBW) was issued, while a general warning is usually included in the prescribing information of Grastims and Interferons. Moreover in the postmarketing databases, such as FAERS and EMEA EudraVigilance, a number of CLS was also reported for Bevacizumab, Cetuximab, Gemtuzumab, Rituximab, and Trastuzumab, with anecdotal reporting related to Alemtuzumab, Efalizumab, Panitumumab, Tocilizumab, and Etanercept.

Therefore, CLS could be observed either as indirect cytokine release following massive immune cell destruction, or as a direct effect of administered cytokines, which represents a solid proof of concept of their etiological role.

More recently, additional data came from the therapeutic experience with monoclonals, starting from R24, an anti-GD3 ganglioside IgG3 murine antibody, and the more common OKT3 (Muromonab), an anti-CD3 murine mAb inducing a strong cytokine release in vitro, and a vigorous CLS in vivo [18,19]. Following the introduction of more sophisticated humanized (Alemtuzumab, Bevacizumab, Daclizumab, Trastuzumab, etc.) and even fully human monoclonals, such as Adalimumab, a number of CLS with a different degree of clinical involvement were observed. In fact, the reduction of immunogenicity obtained by humanization procedures, up to the biosynthesis of fully human mAbs, although remarkably reducing the insurgence of AEs especially related to HR against the mAb glycoprotein backbone structure, could not interfere with direct toxic effects and cytokine releases determined by the triggering of specific cell surface receptors acting on cytokine synthesis and excretion [1,5,20]. In this case, the CLS effect could not be separated by the therapeutic effect, because of the strict relation of both actions with the specific target involved (mainly a cell receptor or an associated component). More interestingly, CLS insurgence correlated with the therapeutic beneficial effect, and therefore has been proposed as a possible predictor marker of efficacy [21].

Among fusion proteins, other than the complex Denileukin-DT, only anecdotal cases of CLS were observed with Alefacept and Etanercept, indicating that the overall drug class profile is rather safe with this respect.

In synthesis, an initial "toxic" effect (a) determined by an external agent, such as a therapeutic agent, may induce a cascade of events involving endothelial injury, cell retraction, and apoptosis, leading to different clinical expressions of CLS. However, the initial event can be also determined by an action on cytokine cell reservoirs (b), either normal or tumoral, targeted by the therapeutic agent. The latter condition, either as principal or adjuvant action, is more related to the administration of biomedicines.

Overall, CLS is a rare, acute, unpredictable adverse event showing cyclicity in the same patient, with intervals from days to decades, albeit stereotyped in the same subject. However, the event carries high morbidity and mortality, although all forms of CLS are usually reversible. But most of all, they can be predicted on the basis of MOA(s) knowledge, and therefore expected during treatment with specific drugs, rapidly diagnosed by the insurgence of prodromic signs, and mitigated by preventive therapy and dosed liquid administration during the acute phase [22, 23].

Cytokine Release Syndrome

Initial unexpected experience with an impressive acute systemic impairment, subsequently related to a massive cytokine release and defined as Cytokine Release Syndrome (CRS), occurred after administration of a murine anti-T monoclonal antibody -Muromonab- documented since its first administrations (1986/87). It appeared as a complex of acute, violent shock-like reactions with relevant Cardiovascular-respiratory, and CNS manifestations associated with a wide spectrum of systemic signs, such as pyrexia, chills, headache, tremors, nausea/vomiting, diarrhea, abdominal pain, and generalized weakness. Most serious and fatal events include cardio-respiratory disorders (dyspnea, bronchospsm, tachypnea, chest pain, respiratory arrest/failure/distress, arrhythmias, cardiac arrest, angina/myocardial infarction), hemodynamic instability with hypertension, hypotension and shock, pulmonary edema, ARDS, hypoxemia, and neuro-psychiatric events. Within days, signals of renal failure (GFR reduction, hyper-creatininemia), and enzymatic signs of hepatotoxicity progressively appear. Manifestations of CNS involvement include progressive impaired cognition, altered mental status, audio/visual hallucinations, psychosis, delirium, and seizures, possibly progressing to aseptic meningitis, encephalopathy, and cerebral edema/herniation.

Local signs of increased vascular permeability and fluid retention may involve vision disorders, up to irreversible blindness, teta/paraplegia, aphasia, and hearing loss. Although some of these signs could be related to typical HR, which may be associated as well, it soon appeared that CRS was associated with an abrupt activation of T lymphocytes and monocytes, consequent to the triggering of specific receptors on these cells, which were over-stimulated by the mAb binding, thus inducing a Cytokine Storm before being killed by the same agent [5].

Candidate targets able to induce CRS were increasingly identified on T cells (CD3, first targeted by Muronomab, CD28), activated T cells (CD25), monocytes (CD52, present also on T, B and NK cells), and on B cells (CD20). It was also clear that the intensity of CRS induction was differently modulated by different agents targeting the same cell surface receptors, by individual patient’s sensitivity, and by the structure of the biomedicine. In the case of mAbs, most violent expressions were observed with murine formulations, which could be subsequently mitigated by the humanization procedures, but could not be avoided even with the most recent fully human monoclonals. In fact, the second dramatic experience unexpectedly occurred with an experimental fully human anti-CD28 mAb (TGN1412) administered in six volunteers in a Phase I trial, leading to serious, prolonged, and life-threatening systemic/multiorgan damage; an hard lesson to be learned in terms of human risk, pre-clinical animal experimentation, and trial procedures [24]. The reaction was characterized by an Acute Phase with headache,
naguse/vomiting, diarrhea, chills, pyrexia, and hypotension, associated with a high level of cytokines in circulation. In a Delayed-protracted Phase multiorgan disorders/failures (MOD/MOF) were observed, mainly as cardio-respiratory and renal impairment, associated with disseminated intravascular coagulation, followed by a prolonged cardiovascular shock, and severe ARDS. From this impressive experience, it became clear and confirmed that: (a) CD28 positive lymphocytes were the major targets and releasers of pathogenetic cytokines; (b) the timing of infusion was crucial for CRS induction; (c) fully human mAbs could not avoid CRS, even in the most serious expressions; (d) the animal models used in pre-clinical analyses were lacking CD28, and therefore could not detect potential CRS induction in humans.

In today clinical experience, due to humanization engineering and mitigation therapeutic procedures, CRS usually appears after the first infusion of inductive agents as general mild/moderate malaise, also identified as Flu-like syndrome (FLS), associated with headache, non infective pyrexia, chills, gastrointestinal distress, musculo-articular pain, and asthenia. However, in a minor number of cases the syndrome may evolve into serious, and sometimes fatal CRS, showing the mentioned severe disorders. Noteworthy, signs of CLS and CRS may be also associated. However, not every clinical sign is present in each affected patient, even when severely expressed, neither they constantly appear in subsequent administrations, nor after introduction of every biomedicine potentially triggering cytokines release. Most importantly, all forms of CRS and CLS are usually reversible and can be timely mitigated by appropriate prophylaxis and therapy, or greatly reduced in frequency by slowing infusion rates.

As for the involved cytokines in CRS induction, the pro-inflammatory compartment is considered the most relevant. Among them, Interleukins – especially IL-1, IL-6, IFNs, and TNFs, as most representative. Interestingly, pyrexia and hyperthermia are more strictly related to IL-1, TNFα and IL-6, the latter being more involved in hyperthermia induction, through mechanisms excluding PGE2 production/excretion [25].

Among biomedicines, CRS seems to be more frequently induced by mAbs directed to T-cells, B-cells and/or monocytes, such as OKT3, Alemtuzumab, Catumaxomab, Gemtuzumab, Rituximab, Tositumomab, although not with the same frequency and intensity [26]. However, in the postmarketing databases, a number of CRS was also reported for Bevacizumab, Cetuximab, and Filgrastim, while anecdotal reporting related to Alemtuzumab, Basiliximab, Canakinumab, Birtumomab, Infliximab, Natalizumab, Trastuzumab, Etanercept, Aldesleukin, Denileukin-DT, and Sargramostim. Under these circumstances, usually high levels of IL-2, IL-6, IFN-γ, and TNF-α are observed [27]. The presence of murine structures in mAbs increases the CRS frequency/severity, possibly due to superimposed HR. It must be stressed that often CRS is not specifically mentioned or warned among adverse events, being frequently included in the Infusion Reaction Syndrome (IRS) phenomenology (see below), or reported by symptomatic features. Therefore, its real frequency may be underestimated.

As expected, CRS expressions are also induced by direct administration of IFNs, Interleukins (IL-1, IL-2, IL-3), and of TNFs, as observed during experimental and clinical therapeutic attempts, thus acting as an additional proof of concept to their etiological role. Furthermore, antibodies -mainly of the IgG type- can directly trigger pro-inflammatory cytokine releases by binding to Fcγ receptors, thus playing as potential inducers of CRS in addition/alternative to the specific receptor binding through the antigen-binding site located in the Fab portion [28].

The overall cytokine release phenomenon does not seem to be associated only with the insurgence of DRAEs and manageable with proper therapy, but in some conditions also correlates with the therapeutic efficacy of treatment. Therefore, the in vitro cytokine releasing capacity of some mAbs, including a direct testing on whole blood, have been offered as predictors both of drug hazard and efficacy [29-31], although with some concerns [32]. In particular, either IFNγ, TNFα, IL-2, IL-4, IL-6, IL-8, and IL-10, or IL-6 alone, have been proposed as markers of the whole cytokine compartment, including anti-inflammatory molecules. In other circumstances such capacity seems to be dependent also from the presence of other co-mediators present on the targeted cells (i.e. EpCAM on tumor cells targeted by Catumaxomab), thus suggesting more complex MOAs related to their induction [33].

An additional complexity derives from the frequent association of HR, showing a high variability of expressions among different biomedicines and patients as well. Nonetheless, the pivotal CRS induction seems to be driven by the biphasic activity of a bio-agent when bound to a specific receptor on lympho-monocytes, consisting in an initial overstimulation of targeted cell functions before the same agent expresses its cytotoxicity on the same target, thus contributing to further increase and diffusion of its bioactive content.

Based on these experiences, recent approaches to new biomedicines’ engineering are directed to avoid the pre-stimulatory phase, by targeting cell surface structures unable to trigger intracellular activating pathways, such as CD25 for Basiliximab, and/or to avoid Fc binding to reduce both CRS and HR, as for Etanercept.

The major problem assessing the real rates of CRS is related to their grading evaluation and description in labels and post-marketing reporting. In fact, only recently NCI has graded CRS expressions in order to attempt a more standardized reporting of the related AEs [34]. Furthermore, most case reports refer only to major events, or to a listing of symptoms frequently associated, but not exclusive of the syndrome. Moreover, the direct assessment of cytokines level is only rarely performed during the event, thus leaving a large margin of uncertainty and possible underestimation of CRS.

**Infusion Reaction Syndrome**

A number of biomedicines induce a complex and rapid reaction when administered intravenously, defined as Infusion Reaction Syndrome (IRS), provoked by various mechanisms of action although not exclusive for this drug class, and by
superimposing immune and non-immune triggering, including true hypersensitivity reactions, anaphylactoid reactions, direct drug toxicity, drug intolerance, and CRS (Figure 1). Consequently, the cohort of symptoms and signs are complex, and include cardiovascular, respiratory, gastrointestinal, dermatologic, constitutional, CNS and psychiatric disorders, among which nausea, vomiting, acute hypotension, pyrexia, chills, bronchospasm, dyspnea, tachycardia, rash and urticaria, are the most frequent. More serious and sometimes fatal events include, anaphylactic reactions, angioedema, myocardial infarction, cardiac and respiratory arrest, syncope, ARDS, and pulmonary infiltrates. Therefore, it is often difficult to individuate the main MOA(s) involved. However, in the case of biomedicines they are frequently observed and ultimately appear to be related to cytokine-mediated reactions of different intensity. In fact, the incidence has been estimated well over 50% in recipients receiving some monoclonal antibodies, such as Muromonab (>90%) and Alemtuzumab (90%), with lower figures for Ibritumomab (≤50%), Ofatumumab (44%), Trastuzumab (40%), Gemtuzumab, Rituximab and Tositumomab (about 30%), Cetuximab (25%), Natalizumab (11-24%), Infliximab (5-22%), Tocilizumab (4-20%), Pertuzumab (13%), Basiliximab (<4%), and for some humanized and fully human agents, such as Bevacizumab and Panitumumab (<1-5%). It must be stressed that more consistent differences in such figures relate to serious adverse events (SAEs), where Alemtuzumab scores for over 40% SAEs, Gemtuzumab for <8%, and the other monoclonals ≤5%. Overall, they include all types of AEs observed during IRS, and generated by every potential acting MOA. However, in a number of cases they have been clearly related to the presence of IgE anti-drug antibodies (ADA) mediating Type I hypersensitivity reactions, such as for Muromonab (29%), Cetuximab (3-13%), and to a minor extent for Tocilizumab, Natalizumab, and possibly with Rituximab and Trastuzumab as revealed by Type I skin reactivity [35]. In the case of Basiliximab, the specific IgE response was directed exclusively against its idiotypes [36]. In some instances the IgE-ADA were preferentially directed to the oligosaccharides component of the administered mAb, which could be raised even before its infusion against other cross-reacting antigens. Noteworthy, in the case of Omalizumab, Type I reactions appeared to be directed to an excipient (polysorbate), as in the case of some Epoetins [1, 2, 37-39].

At present, nine mAbs (Alemtuzumab, Cetuximab, Gemtuzumab, Ibritumomab, Muromonab, Panitumumab, Rituximab, Tositumomab, Trastuzumab), and one fusion protein (Denileukin-DT) have a BBW on IRS, thus indicating the major role of IRS among DRAEs for these agents. Notably, these biomedicines are not directed to a unique target, although the majority points to cell surface structures expressed on T-cells, B-cells, and monocytes, such as CD3 (Muromonab), CD20 (Ibritumomab, Ofatumumab, Rituximab), CD30 (Brentuximab), CD33 (Gemtuzumab), CD52 (Alemtuzumab), CD80/86 (Belatacept), and α4-integrins (Natalizumab). Moreover, other bio-agents inducing IRS are directed to epithelial cell components, such as EGFR (Cetuximab, Nimotuzumab), and HER-2 (Pertuzumab, Trastuzumab), or to endothelial soluble factors (VEGF), such as Bevacizumab and Afiblercept. Similarly, they do not pertain to the same structural class, since they include not only mAbs and fusion proteins, but also some cytokines (IL-1, IL-2), and respective cytokine analogues, such as Anakinra and Aldesleukin developing systemic HR as well [40].

A general and relevant characteristic of IRS caused by these agents is their appearance with the very first infusion, and the tendency to decrease over time with subsequent administrations. The phenomenon has not been explained, but is being attributed to an acquired tolerance, or to a sort of tachyphylaxis-like effect. In oncological treatments with relevant tumor burdens, mostly when circulating and consisting of cells with high-yield of cytokines (lymphocytic leukemias), such effect is attributed to the drastic reduction of the tumoral mass releasing both toxic and bioactive molecules, which better explains the violent and life-threatening manifestations, and the rapid reduction of symptoms in subsequent administrations.

As previously reported, IRS are most probably produced by a complex intervention of different MOAs, including non-immune and immune reactivity, and even by the existence of pre-existing antibodies against murine and human antigens, present in a significant number of normal individuals, cross-linking with the respective analogs inserted in the mAb or FP structures. This may also explain the insurgence of immune-mediated IRS at the very first administration. In this respect, not only the proteic backbone is important, but also the glycosylation, either as voluntarily associated during mAb structuring or as accidental, raising specific antibodies able to induce HR as well. In fact, the saccharide content of these molecules is also immunogenic, with relevant differences in inducing anti-glycoprotein antibodies even for minimal structural variations, as experienced in the recent production and validation of biosimilar Epoetins and growth hormones [41-43].

A typical accidental example of unwanted glycosylation was experienced during the production of Cetuximab, initially performed in the supportive Sp/0 murine cell line having a sialic acid (N-glycolyneuraminic acid), which was found in the mAb molecule as a consequence of the manufacturing process, due to the presence of α-1,3-galactosyl transferase in this cell line. Such murine sialic acid raised specific antibodies giving rise to a number of DRAEs, which were subsequently avoided by producing Cetuximab in the CHO cell line lacking the transferase necessary for the sugar binding to the mAb.

Fusion proteins indicated for infusions elicit a minor and mild number of IRS, such as with Belatacept (5%) thus indicating the crucial role of the absence of the Fc fragment in these agents, both lowering the proteic content and the associated saccharides in inducing immune-mediated DRAEs.

Overall, IRS are more frequent during the first infusions, tend to drastically reduce their severity with subsequent administrations, are usually controlled by prophylactic medications, and are mitigated by slow infusion ratings. Nonetheless, in a small number of cases IRS can be serious and fatal. Most IRS related to biomedicines are caused by CRS, although they may be associated to HR and appear with superimposable symptoms and signs, especially with those related to anaphylactic/oid type. However, the latter are
Tumor Lysis Syndrome

The syndrome was first described in 1929 in patients with chronic leukemia, and manifested with acute renal failure associated with hyperkalemia, hyperphosphatemia, secondary hypocalemia, elevated LDH, and pyrexia [46].

Typically, Tumor Lysis Syndrome (TLS) is observed in patients with high load tumors, more frequently hematologic with a high number of circulating cells with elevated turnover, undergoing rapid massive spontaneous or induced lysis. Their destruction frees a considerable quantity of ions and toxic metabolites affecting renal function, and the subsequent development of a serious multisystem organ failure [47,48]. The increasing electrolyte imbalance and accumulation of toxic byproducts progressively induce MOD/MOF, inducing cardiac, muscular, hepatic, and neurological dysfunctions, with a wide spectrum of severity, up to fatal evolution.

Spontaneous TLS mainly occurs in CLL, high-grade lymphomas, and acute leukemias [49], but also in solid tumors with high cell turnover [50], or with peculiar cytotypes [51].

Secondary TLS may follow aggressive chemotherapy and radiation therapy, less frequently after heavy corticosteroids administration, and after treatment with various anti-tumoral biomedicines. This has focused new attention on the involved MOAs. Other than in hematological proliferative disorders, secondary TLS has been observed in patients with solid tumors (hepatocarcinoma, high-grade neuroblastoma, renal cell carcinoma, gastrointestinal tumors, pancreatic neuroendocrine tumors, melanoma), and in particular in neoplasms with high turnover showing high sensitivity to specific treatments.

The central MOA seems related to the presence of high levels of potassium, phosphorus, and nucleic acids in neoplastic cells, which undergo abrupt lysis causing hyperuricemia, mainly affecting the renal function. Secondary hypocalcemia, the second life-threatening consequence of TLS, appears related to calcium binding to the circulating elevated amount of phosphorus determined by tumor lysis. The consequent Ca/P imbalance produces arrhythmias, and hypotension, possibly leading to cardiac failure and arrest.

Interestingly, high levels of hyperphosphatemia are observed after secondary TLS, in comparison with spontaneous TLS where there is a higher re-usage of phosphates by newly growing tumor cells, which is inhibited by the prolonged effect of the treatment in secondary TLS.

More recently, a Prodomic TLS was identified, showing an increase of electrolytes associated with signs of renal injury 3-7 days after cytotherapy initiation. This phase may develop cardiac dysfunctions (arrhythmia), creatininemia, and neurological disorders (seizures), up to rare cases of sudden death. Importantly, proper therapy (anti-uric, hemodialysis) may prevent the progression of the prodromic phase into clinical TLS.

A Laboratory TLS is also recognizable, in the absence of relevant clinical signs, which is of relevant importance in foreseeing and preventing its evolution into full TLS. [46]

The experience with biomedicines and other recent drug classes (protein kinase inhibitors, proteasome inhibitors) have focused new attention on the syndrome, indicating that TLS susceptibility is not restricted to a particular drug class, nor to a specific target, but most probably to the tumor specificities (high mass, elevated cell turnover) and/or other individual specificities, such as renal or cardiac clinical conditions antecedent to anti-tumor therapy initiation. But most of all, the particular violence of TLS has been related to the high efficiency of target destruction and triggering effects exerted by the new anti-tumoral biomedicines.

Following previous dramatic observation after Muromonab (anti-CD3) administration, TLS of various severities have been mostly experienced and even predicted with Alemtuzumab (anti-CD52), Brentuximab (anti-CD30), Gemtuzumab (anti-CD33), Iplimumab (anti-CTLA4), Ofatumumab and Rituximab (anti-CD20), because of their high efficiency in massive tumor cell destruction. Recently, a fatal case of TLS following Trastuzumab (HER-2) monotherapy in metastatic breast cancer has been reported [Figure 1; 52].
In some cases, such as with agents interfering with CD3, and CD20, the destructive phase typical of TLS could be preceded by an overstimulation of the cell targets caused by the mAb binding to their specific cell surface structures, in targets particularly rich of cytokines. This effect is peculiar of secondary TLS following treatment with biomedicines, which may induce an acute CRS followed by a classic TLS in sequence, and even a typical CLS followed by CRS and TLS, as in the case of Alemtuzumab administrations, as incoming Shock Waves induced by different bursting MOAs.

Almost 90% of secondary TLS appear during the first cycle within two days after treatment and seem to correlate with a massive liver metastatic involvement, and with a higher rate of mortality in solid tumors, compared to hematologic neoplasms, thus indicating that massive liver metastatic destruction may represent an additional risk factor for development of dramatic TLS [52,53]. Another high-risk situation for secondary TLS has been evidenced in children with hematologic (B-NHL) malignancies (25%), including treatment with Rituximab associated with COP chemotherapy, which could be favorably influenced by preventive therapy [54]. Finally, even stimulatory factors (G-CSF) may induce secondary TLS, with high rates of leukolysis [55].

Overall, TLS remains a rare event, due to increased knowledge of triggering conditions, prodromic signs, and to proper vigilance and prophylaxis [56]. The higher frequency of TLS in cytokine-rich targets, such as leukemia and lymphoma, together with an increased observation of secondary TLS following treatment with biomedicines targeting such cells, and the rare association of clinical signs of CRS with typical TLS features in wave-like acute cascades, suggests that cytokines play an additional relevant role in TLS expressed during anti-neoplastic therapy with biomedicines with highly destructive efficiency, especially in the induction of additional extra-renal MOFs. Therefore, particular efforts are being made to build up new therapeutic agents incapable of inducing the prodromic overstimulation of the targets before causing their destruction, along the line suggested by Basiliximab engineering [1].

**Systemic Inflammatory Response Syndrome**

The Systemic Inflammatory Response Syndrome (SIRS) is characterized by an acute progressive inflammatory reaction, triggered either by infections or by non-infectious stimuli, such as trauma, ischemia, and specific therapeutic interventions. Major signs include body temperature imbalance (pyrexia or hypothermia), quantitative leukocytes abnormalities (leukocytosis or leukopenia), and increased cardio-respiratory rates. The diagnosis of SIRS is based on etiopathogenetic results and the presence of at least two of such major clinical signs, i.e. temperature imbalance, (<36 or >38 °C), tachycardia (>90 bpm), tachypnea (>20 bpm), and quantitative leukocyte abnormality (<4,000, >12,000 mm3) [57,58]. More recently, SIRS and CRS has been reported as a consequence of administration of genetically modified and even unmodified T-lymphocytes, which follow their expansion in vivo after infusion, and in association with TLS [59,60].

Infectious SIRS may show or progressively evolve into sepsis, septic shock, and MOD, as defined in 1992 by the American College of Chest Physicians (ACCP).

Non-infectious SIRS usually develop MOD and failure, such as renal and liver failure, gastrointestinal bleeding, anemia, deep vein thrombosis, disseminated intravascular coagulation, hyperglycemia and electrolytes imbalance. Overall, SIRS occurs in about 35% of acutely hospitalized medical patients, and highly influences their mortality [58]. Initial triggering has been related to endotoxins, anaphylaxis, complement activation, endothelial vascular injury, CLS and CRS, the latter two being especially induced by biomedicines administrations. In fact, most of these events are considered potential pro-inflammatory cytokine releasers, and major responsibility for SIRS induction and progression has been attributed to TNF-α, IL-1, IL-6, IL-8, and possibly to IL-17 [61]. Macrophages, monocytes, mast cells, endothelial cells, in addition to lymphocytes, are involved in this process.

Cases of infectious and non-infectious SIRS have been reported, either in controlled studies or in the post-marketing reporting, for Adalimumab, Alemtuzumab, Basiliximab, Bevacizumab, Catumaxomab, Cetuximab, Gemtuzumab, Infliximab, Natalizumab, Rituximab, and Trastuzumab, as an average of over 100 cases/each reported. Less frequently, SIRS have been observed during treatment with Certolizumab, Daclizumab, Golimumab, Ibritumomab, Omalizumab, and Palivizumab (<100 cases/each). Occasional/anecdotal reportings include Absciximab, Belimumab, Brentuximab, Canakinumab, Denosumab, Eflazimub, Golimumab, Ipilimumab, Ofatumumab, Omalizumab, Panitumumab, and Tositumomab (≤ 30 cases/each).

Among fusion proteins, SIRS are less frequent, and have been observed during treatment with Abatacept, Alefacept, Rilonacept, Filgrastim, and Romiplostim, with higher incidences for the latter three agents.

Cytokines and their analogs can also induce SIRS at lower rates, such as during experimental and therapeutic administration of IFNs, Anakinra, Darbepoetin, Denileukin-DT, Anakinra, and Oprelvekin treatment. The majority of reported SIRS are classified as infectious, while <10% are non-infectious which can be attributed to a direct involvement of the drug in use, while part of the former ones can be ascribed to indirect immunosuppressive effects of the biomedicine in study, unmasking or enhancing infectious conditions. In particular, non-infectious SIRS are associated with a more direct involvement of cytokine unbalance leading to CRS-like situations, while the larger group of infectious SIRS have pathogenetic routes more related to direct toxic effects, without exclusion of concomitant interventions of CRS.
phenomena, especially when in the presence of cytokine-rich targets. The evolution of CRS-related SIRS can be impressive and rapid, even without infectious clinical complications [45]. In fact, a number of bacterial products can directly stimulate cytokine production, thus exerting an additional impact during SIRS, and even apply for a reliable prognostic tool during their development [62]. In particular, IL-4, IL-6, IL-8 and TGF- β have been proposed as biomarkers in non-infectious (traumatic) SIRS. However, not only increased levels of pro-inflammatory cytokines have been observed, but also a lowering of anti-inflammatory cytokines eventually improving the bursting of the former ones [63]. Moreover, the intervention of exogenous cytokines (i.e. Grastims) on underlying pro-inflammatory cytokine concentrations (i.e. TNF-α) may interfere and produce different clinical responses and DRAEs physiognomy, possibly causing synergistic unwanted effects. By contrast, low doses of exogenous rhG-CSF (Filgrastim) although inducing an increase in neutrophils, attenuated their phagocytosis and oxidative-bursting to such extent the overall result proved beneficial during both infectious and non-infectious SIRS [64].

As expected, when multifactorial mechanisms either drug-related, disease-related, or occasionally overlapping are concomitantly interacting, the recognition of the SIRS pathogenetic roots becomes difficult. Even unexpected interferences due to specific immune stimulations (anti-influenza vaccination) may modify or aggravate the insurgence of SIRS, and/or the underlying autoimmune disorders [65].

Taken together, overall post-therapeutic SIRS appear to be underestimated, due to the difficult distinction among clinical signs derived from concomitant infections, and from non-infected CLS/CRS events in the same patients. Nonetheless, early and proper recognition of SIRS together with early therapeutic intervention can significantly lower the most severe outcomes of the disease [66].

Macrophage Activation Syndrome

The Macrophage Activation Syndrome (MAC) is more frequently observed in Systemic Juvenile idiopathic Arthritis (SJIA) and in other rheumatic disorders, as a serious life-threatening complication characterized by intense macrophage hemophagocytic activity, mostly evident in bone marrow, and leading to thrombocytopenia, pancytopenia, coagulative disorders, hepatic injury with hepatosplenomegaly, encephalopathy, increased ferritinemia, and elevated non-remitting pyrexia. Additional laboratory disorders include high levels of triglycerides and LDH, hyponatremia, and hypoalbuminemia. MAS is also defined as Hemophagocytic lymphohistiocytosis (HLH), and is part of the so-called Hematophagocytic Syndromes (HPS), with frequent acute and dramatic evolution and a high rate of mortality. MAS is also a complication of SLE, including pediatric SLE, Kawasaki disease, autoimmune-inherited diseases (CAPS, associated with NLRP3/CIAS1 mutations), lymphomas (mainly NHL), and may follow acute EBV, CMV, and Herpes Virus infections [67-71]. A primary inherited form of the disease is also known as Chédiak-Higashi Syndrome. Quite recently, an additional genetic mutation in the ADP binding domain of NLRC4 has been found in a MAS-like case, associated with an enhanced pathogenic production of IL-1 β, IFN2a and particularly of IL-18, thus suggesting a major role of these cytokines in the expression of the syndrome [72]. However, the most relevant abnormalities in MAS patients concern the functional impairment of NK and of cytotoxic T cells [73]. The situation leads to a persistent antigen-driven cell activation, mainly into the T cell compartment, causing again a consistent production of cytokines, which act on macrophage proliferation and function.

Infectious MAS seem to be related to the specific action of the two major viral agents involved (CMV, EBV), through yet unclear MOAs.

Non-infectious - inherited and idiopathic - forms indicate possible genetic NK and cytotoxic T cells defects, some of which related to the perforin-mediated cytotoxicity. More stringent evidence is related to the presence of a number of factors influencing the macrophage activity/function during clinical MAS expression, such as M-CSF, IFN-γ, MCP-1, and of macrophage-derived cytokines (IL-6, IL-12, IL-18, and TNF-α). Other T-cell derived (IL-2, IL-2R, IL-1) products have been also evidenced; in particular, IL-1 is not always detectable at elevate levels, but seems to play a relevant role in MAS, since treatment with an IL-1 antagonist (Anakinra) has beneficial effects on MAS expression, thus indicating a crucial role of IL-1 (IL-1β), both in MAS and SJIA [74,75].

As for drug-induced MAS, it is known that acetylsalicylic acid, and other NSAIDs, gold salts, sulfasalazine, and more recently biomedicines with a high capacity of cytokine release can provoke its insurgence. In particular, TNF-α seems to be involved in dyscoagulative disorders occurring along with MAS expression. Quite recently, innate immune signals through adjuvant repeated stimulation of Toll-like receptor 9 (TLR9), usually raising tolerant signals in animal models, could lead to MAS associated with IFN-γ and IL-12 increase [76], thus posing intriguing new interpretations on the pathogenesis of MAS, both in infectious and non infectious forms. However, MOAs related to the use of biomedicines are not always a direct consequence of cytokine release mediated by the therapeutic agent. For example, IL-18 seems to play an indirect role in MAS, as well as in a number of autoimmune diseases, because of its involvement in the production of IFN-γ, from T-cells and NK cells, implicated in major MAS symptomatology and injury [77].

Overall, MAS appears to be another feature related to a selective cytokine release particularly involving macrophage activity and function, although other mechanisms may be involved as well. In the case of Alemtuzumab, a reactivation of EBV and/or CMV has been also observed. Therefore, and in more general terms, immunosuppressive effects of administered drugs may induce an indirect infectious MAS activation, thus enlarging the possibility of its induction to other agents not directly capable of triggering CRS. Contrasting evidence of beneficial vs inductive MAS effects from some agents, such as Anakinra and Tocilizumab,
suggests more heterogeneity among the involved MOAs in different clinical conditions where the underlying disease may make the difference [78,79]. Additional rare cases of MAS have been reported in the post-marketing settings during Etanercept, Rilonacept, and Filgrastim administrations.

- Noninfectious IRIS is usually experienced in rheumatic diseases (RA, SLE), in other autoimmune disorders (thyroiditis, GBS), and in granulomatous disorders (sarcoidosis), mainly treated with anti-TNFα agents, where cutaneous and non-cutaneous onsets and worsening may combine/exacerbate the preexisting pathology. In other instances a new autoimmune onset may be observed in association of IRIS outcomes in infectious patients. Notably, lymphoma incidence is also increased during IRIS in HIV patients [91-93].

- Local IRIS in the CNS has been identified as a peculiar form of Progressive Multifocal Leukoencephalopathy (PML), or IRIS-PML, soon after therapy cessation with Natalizumab in MS patients, associated with JCV reactivation [94]. The pathogenesis of IRIS is ultimately based on a rapid and often exuberant immune-inflammatory response of the host to resident microbial antigens or on an aspecific homeostatic rebound. However, the specific MOA(s) involved at cellular and molecular level are less clear. During IRIS a consistent increase of CD4+ T lymphocytes with a variable association of CD8+ T cells (particularly rich in HIV-associated sarcoidosis), active macrophage infiltration and necrosis are usually observed, together with signs of infective reactivation, such as mycobacterial lymphadenitis, recurrence of opportunistic pulmonary infection, and viral reactivation. During anti-HIV therapy, IRIS appears when CD4+ cells rapidly increase, and in a more complex way after discontinuation of more extended immunosuppression, either with chemotherapy or with anti-tumor or anti-rheumatic monoclonal antibodies. In fact, during immune post-therapeutic reconstitution, cell recovery kinetics is dissimilar not only among different classes and subclasses of leukocytes, in particular for lymphocyte subtypes, but also in their peripheral redistribution. For example, memory CD4+ cells appear to anticipate their recovery with respect to naïve T cells, even for months, while Treg cells appear compromised longer, while a consistent production of inflammatory interleukins (IL-2, IL-6, IL-12, IFN-γ) interferes with the whole scenario. This clearly indicates the rapid establishment of an unbalanced immune condition, where pro-inflammatory cytokines burst up, in the absence of adequate controls from Tregs and possibly from other downregulatory signals, including the intervention of anti-inflammatory cytokines (IL-4, IL-10).

In the case of local IRIS-PML, the rebounded situation shows different features. The brain histology showed an extensive infiltration of T cells, particularly CD8+ lymphocytes, B lymphocytes and plasma cells, together with a low number of JCV-infected cells, as compared to classical PML usually encountered in adult and pediatric HIV-infected patients [95] In fact, the number of T cells in IRIS-PML was found to be up to 9 times higher than in PML, while B-cells and plasma cells were practically absent in the latter, showing instead a higher number of JCV-infected cells [94]. Taken together, an unbalanced recovery after therapy discontinuation, mainly because of the impairment of the Treg compartment and the overstimulation of pro-inflammatory cytokines, justifies most of the clinical immune rebound signs, both in infectious and non-infectious situations. However,
other factors may well be engaged. Among these, VEGF signals seem to be involved, since anti-VEGF therapy (Bevacizumab) showed to be effective in controlling some TB-IRIS granulomatous infections [96]. A number of TB and fungal IRIS have been also observed after TNF-α antagonists (Infliximab, Adalimumab) discontinuation [97-100]. Notably, a concomitant recovery of reactivity to tuberculosis was observed together with the reappearance of granulomatous pulmonary lesions. In some life-threatening cases mAb therapy was reintroduced with beneficial effect on TB-IRIS, thus confirming the link between immunosuppression and IRIS rebounding insurgence. Overall, the immune over-response often appears to be of the granulomatous type, although the predominant cell component is represented by dysregulated expanding CD4+ lymphocytes, which is indicated as the major supporter of IRIS, possibly associated with an hyper-responsiveness of the innate immunity to T cell help [86]. Interestingly, the rebounded inflammatory response tends to be preferably localized, even in those infections (mycobacterial) that are typically disseminated after immunosuppressive therapy, and exuberant even before normal T cell levels appear into circulation [101], suggesting a protective confining role of IRIS-mediated granulomatous intervention.

Unmasking infectious and local IRIS have been observed after treatment with other biomedicines, such as Efalizumab (PML), Ibrutinumab, Ustekinumab, and Etanercept, including the administration of Grastims in non-HIV patients [102], while anecdotal cases (about 10 cases each) were reported in the postmarketing observation for Abatacept, Alefacept, Aldesleukin, IFNs, Iplimumab, Rituximab, Romiplostim, and Filgrastim. Natalizumab, which is responsible of over 1000 reports in the postmarketing databases caused the highest number of PML among biomedicines, but could also unmask serious cerebral infections [103, 104].

It must be stressed that not all immunosuppressive drugs, as well as biomedicines with immunosuppressive effects are able to induce IRIS after discontinuation, thus indicating that more complex yet unknown MOAs are most probably involved in the insurgence of the syndrome.

Immune-Related Adverse Event Syndrome

Quite recently, another and intriguing reaction has been observed during Iplimumab administration, defined as Immun-related or Immune-mediated Adverse Events Syndrome (IrAES or ImAES) caused by the action of the anti-melanoma mAb as a potent immune response inducer, especially on its effector functions. Iplimumab is directed to CTLA-4 (CD152) cell surface antigen and shows promising results on Stage III-IV unresectable malignant melanoma. Its MOA is substantially different from all other anti-tumor biomedicines, since it does not target tumor cells, but induces a high boost of immune reactivity, including specific tumor aggression in combination with a vaccine or with chemotherapy. Blockade of CTLA-4 signaling prolongs T-cell activation and restores T-cell proliferation, by increasing IL-2 secretion and IL-2R expression, thus amplifying T-cell-mediated immunity and an antitumor immune response. It is believed that the major role of CTLA-4 under physiological conditions relates to maintenance of self-tolerance and inhibition of autoimmune reactions. However, the inhibition of CTLA-4-mediated signals has produced a number of serious and fatal multi-organ inflammatory-like processes of any grade, such as dermatitis (slightly over 40%), enterocolitis (about 30%), endocrine (4-8%) inflammatory disorders, and hepatitis (2-4%), driven by a massive activation of T cells, in over 64% of treated patients [105]. Kidney functional involvement is a more rare event and may appear also as acute granulomatous interstitial nephritis demanding early diagnosis and treatment initiation [106].

Most common signs of gastrointestinal involvement are diarrhea (20-30%), followed by abdominal pain, nausea, vomiting, associated with pyrexia and fatigue, and supported by histopathological findings such as leukocyte and plasma cell infiltrates, crypt abscesses and mucosal ulceration, mainly located in the lower part of colon. Most IrAES reactions are mild to moderate, but in about 1% of cases they can be serious and fatal, and are already triggered during the induction phase, although delayed IrAES have been observed even months after therapy discontinuation. Endocrine insufficiency disorders mainly involve thyroid, hypophysis, and adrenal glands. In a recent retrospective study on 256 patients, hypophysitis (8%) and hypothyroidism/thyroiditis (6%) rates were better evaluated, showing frequent irreversible damage of endogenous pituitary hormone secretion [107]. However, cases of hyperthyroidism have also been observed, associated with typical systemic signs. In addition, a minor number of sensory and motor peripheral neuropathies, GBS, and MG were also observed. Quite recently, atypical neurologic disorders, such as chronic inflammatory demyelinating polynueopathy, and transverse myelitis have also been reported [108]. During Iplimumab treatment TLS may develop as well, although in a limited number of cases. As for the onset timing trends, dermatologic IrAES appear within 2-3 weeks, gastrointestinal and hepatic disorders within 6-7 weeks, and endocrinopathies in about 9 weeks. Usually these events are reversible in few weeks after therapy discontinuation, except for some endocrinopathies, which can persist or become irreversible [109,110]. At ocular level, uveitis, scleritis, and conjunctivitis have been observed. Most serious adverse expressions relate to Stevens-Johnson syndrome, toxic epidermal necrolysis, and complicated rash at skin level, liver toxicity associated with encephalopathy, and adrenal crisis requiring proper and prompt management [111]. Notably, IrAES induced hepatitis is preceded by an asymptomatic worsening of functional tests, and can be early recognized and monitored.

Overall, the clinical features expressed during therapy with Iplimumab can be referred to its specific MOA, and appear to be dose-related and reversible upon treatment discontinuation. However, the management of IrAES -based on corticosteroids and supportive care according to the main organ(s) involved by the immune-inflammatory aggression- is particularly important, since Iplimumab discontinuation may
be at risk of tumoral bursting. Therefore, the risk of serious and fatal complications, both as IrAES and tumor expansion remains a real concern. Because of the existence of life-threatening cases non-responsive to high doses of corticosteroids, it has been found that Infliximab can be beneficial in such critical situations, especially for gastrointestinal IrAES [112]. Moreover, initial investigations are focusing on the individualization of predictive genetic and laboratory markers of efficacy such as the decrease of LDH, CRP, and FoxP3/Tregs, together with an increase of ALC levels over the course of treatment, to better address therapy [113, 114].

Conclusion and perspectives

The knowledge about the mentioned Systemic Syndromes has grown rapidly after the introduction of biomedicines, because of their expansion and the possibility of better understanding the underlying MOA(s), since these new agents hit specific cell targets expressing specific cell functions. MOA identification attempts have shown, among other characteristics, that these syndromes and more in general the induction of DRAEs with this drug class can either follow their administration or treatment discontinuation, with selective or generalized consequences. They include toxic effects, cell recruitment and activation, and cell lysis, leading alternatively or in sequence to immune activation, immunosuppression, and immune rebound according to the administered agent, its route of administration, and to associated therapeutic interventions (Figure 1). In particular, the pre-activation of individual cell targets, before the pharmacologic action of the administered agent brings the targeted cells to suppression or death, the hyperactivation and the inflammatory/immune rebounds after therapy discontinuation or after the blockade of inhibitory immune signals, are the most peculiar aspects of DRAEs induced by Biomedicines. Most importantly, these phenomena are independent from true immune AEs, such as the induction of ADA, HR, and LOR consequences, although frequently appearing in concomitance.

Although all systemic syndromes may be considered infrequent AEs, they can exert serious and life-threatening features in about 10% of cases. However, the renewed attention has also produced particular interest in their prompt diagnosis and prevention, so that they can all better be individuated and controlled.

Taken together, most systemic syndromes induce or end up with a major cytokine imbalance, responsible of most of the clinical signs. In particular, such condition can be related to: (a) massive cytokine release, (b) cytokine dysregulation, (c) cytokine rebound. The first situation is best represented by CLS and CRS induced by different agents, and expressed by different and impressive clinical features; the second condition can be assimilated to MAS and SIRS, in which other MOA(s) may superimpose. The third situation seems best represented by IRIS, and by IrAES, although with different mechanisms and different clinical expressions. Finally, in the case of IRS a number of immune and non-immune MOAs are in play, including the cytokine storming with a variety of differential expressions, which are difficult to assign to specific pathogenetic roots.

IrAES shed new and unexpected light on a complex situation in which the blocking of an inhibitor immune receptor, CTLA-4, has produced a sort of “breaks off” effect on the immune system, with consequences that must be better investigated before attempting to locate them in a precise typology. Importantly, such events, leading to various MOD/MOF, can be associated with TLS and CRS-related signs, thus further enhancing the autoimmune attacks to specific organs. Fortunately, most reactions are controlled by corticosteroids, which seem not to interfere with the anti-tumor therapeutic efficacy of the biomedicine.

It is not easy to differentiate among the listed systemic syndromes, nor between them and a number of concomitant factors inducing AEs during treatment, although the investigated physiognomy observed during administration of some biomedicines has improved their differential understanding. A typical example of a complex concomitance of events is IRS, where hypersensitivity reactions, cytokine releases, drug toxicity, anaphylactoid reactions, and idiosyncratic reactions are interplaying and favoring rapid cytokine imbalances.

The crucial role of cytokines is not only important for the development of adverse events, but seems to be related to the therapeutic efficiency of some biomedicines, since a strict correlation between CLS/CRS and drug efficacy has been observed, to suggest a predictive role of their insurgence on clinical response. In particular, the pro-inflammatory group of cytokines carries the major responsibility, and therefore IL-1, IL-2, IL-4, IL-8, IFNγ, TNFα, and primarily IL-6, have been proposed as markers of the whole cytokine compartment. In fact, anti-inflammatory cytokines (IL-4, IL-10, IL-13) are also unbalanced in such situations, and may have a crucial effect in downregulatory control of the syndromes, although less information is available on their role on both DRAEs and therapeutic predictability. The whole scenario is deeply influenced by patients’ individual sensitivity to administered drugs. In fact, not all symptoms typical of each systemic syndrome are present in the same patient, while a periodical cyclicity of signs may be observed over time in the same patient, such as during CLS events.

Taken together, it becomes evident that in all depicted syndromes the role of cytokine imbalance appears as the core of clinical consequences offering a wide spectrum of signs and evolution, from mild disturbances to life-threatening conditions. Such pivotal event can be induced by different actions, and associated/preceded by other mechanisms leading ultimately to a cytokine dysregulation status, which may develop towards different directions/expressions identified in the mentioned Systemic Syndromes, due to the prevalence of single cytokines expression and/or target exclusivity (i.e. macrophages for MAS; endothelia for CLS), their localization (brain for RPLS and PML), or generalization (CRS, SIRS, IRIS) due to multiple targets/organs involved, or because of the association with preceding/concomitant events inducing massive toxicities (TLS), or autoimmune multiple.
aggressions, due to the “breaks off” effect on inhibitory immune signals (IrAES). On these bases, a unifying pathogenetic hypothesis based on a dynamic cytokine dysregulation or a Cytokine Dysregulation Syndrome (CDS), as the mother of all mentioned Systemic Syndromes is depicted in Figure 2, with the aim of stimulating a more straightforward research and debate.

Lessons have been already learned and important indications have been acquired, such as the limitedness of animal models in predicting cytokine storms, and the possible better support from combined in vitro testing on the synthesis and the release of cytokines during therapy. Furthermore, particular attention was already drawn to avoid pre-activation of cell targets before their blocking/destruction, a strategy already applied to new biomedicines’ research. Similarly, the identification of more and more selective targets, are being searched to avoid generalized inhibitions and unexpected consequences, such as those experienced with anti-CD3 monoclonals. Finally, the recent experience with IrAES as a consequence of the “breaks off” effect on immune regulatory signals, further suggests more selectivity of intervention also on inhibitory pathways, to avoid dramatic immuno-inflammatory bursting experienced, such as with the anti-CD152 Ipilimumab. A better understanding on the risk/benefit balance of subcutaneous administrations with biomedicines is already under investigation with the aim of reducing systemic events as well. Finally, when compared to chemotherapeutics, and non-biological immunosuppressors, biomedicines offer less toxicity and more triggering effects, which need to be more carefully avoided in future molecule engineering and promptly controlled at bedside. Increasing attention is devoted to potential therapeutic use of anti-inflammatory cytokines.

For all these purposes, the individuation of affordable markers both for CRS and HR reactivity, predictability and early diagnosis are demanded. Furthermore, a more astute approach to cytokine assessment must be put in play. In fact, these molecules act in a complex twine of interactions, where pro- and anti-inflammatory signals compete in the expression of CDS. Such contrasting signals are either dependent on different soluble bioactive agents, but also on the same molecules according to local conditions and mutual interferences in a dynamic network, which must be explored according to systems biology approaches. Moreover, in some instances the cytokine level appears within the normal range, but a specific antagonist therapy has remarkable beneficial effects (i.e. Anakinra for IL-1 in MAS), thus suggesting a pathogenetic role beyond a simple excess of production of a single molecule, or a lack of homeostatic anti-inflammatory response. This complexity may explain why by measuring single cytokines at fixed points of CDS expressions pro-inflammatory levels have been found either elevated or normal or, in other circumstances, their high level has persisted after CDS mitigation and recovery. Kinetics of both pro- and anti-inflammatory compartments are most probably more important than absolute levels of single components in play.
References


Macrophage activation syndrome in autoimmune diseases. *Int Arch Allergy Immunol* 2010;153:109-120.


