Early versus late immune mediated inflammatory diseases

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Abstract

Immune mediated inflammatory diseases (IMIDs) are life long conditions that cause substantial morbidity and disability. Though increasingly common and intensely studied, the cellular and molecular mechanisms underlying their pathogenesis are still unclear. Despite this incomplete knowledge, it is becoming increasingly evident that IMIDs evolve over time, not only from a clinical perspective but also a pathophysiological one. Evidence is accumulating that the events responsible for inflammation and damage in the target organs are not necessarily the same during the evolution of the IMID, and that the immune response evolves in parallel with the clinical manifestations. This has crucial implications for therapy because immunomodulatory interventions aimed at early pathogenic events may no longer be effective when these events have changed due to a different composition of the immune response. Therefore, it is crucial to better understand why and how the IMID associated immune abnormalities evolve over time, so that time-dependent therapies may be rationally implemented for an improved clinical outcome. (Acta gastroenterol. belg., **2011**, 74, **548-552**).

Key words : autoimmunity, autoimmune diseases, immune mediated inflammatory diseases, inflammatory bowel disease, Crohn's disease, ulcerative colitis.

The concept of early and late disease

Immune mediated inflammatory diseases (IMIDs) are frequent ailments of Western societies and are becoming increasingly common in other evolving societies, like those of the Asian Pacific rim, as they adopt westernized life styles. The medical, social and economical burden of these conditions, such as asthma, rheumatoid arthritis, inflammatory bowel disease (IBD), psoriasis, multiple sclerosis, etc., is enormous not only because of the severity of the symptoms, but more so because of their characteristically chronic nature that is essentially life long. It is becoming increasingly apparent that IMIDs display distinct phases while they evolve during the life of the patient, as indicated by differences in severity and type of symptoms, response (or not) to therapy, and clinical outcome. This variability has led to the notion that IMIDs display "early" and "late" phases of evolution. While these phases are certainly real, they are not easy to readily recognize or classify because they are part of an evolutionary continuum. Consequently, what may be called "early" and "late" disease is often confused with acute and chronic, or paediatric and adult disease. This creates further confusion because acute flare-ups can obviously occur in advanced stages of IMIDs, and children with a short history of an IMID can display chronic manifestations of their disease as much as an adult can have his or her first IMID manifestations late in life. Further complicating the definition and understanding of early *versus* late IMIDs is the fact that the amount of information on the pathogenesis of each condition that directly compares similarities or differences between the two stages is quite imbalanced : fairly limited data are available on humans with early IMIDs whereas most experimental studies have been performed in animal models of acute IMIDs ; the opposite is true for late disease, as humans are usually studied when the disease is already well established and chronic, while animal model of chronic IMIDs only recently began to receive due attention. These considerations apply to all IMIDs, but for the purpose of the following discussion we will refer primarily to IBD as a prototype of an early *versus* late disorder.

Time dependent evolution of the immune response

The fact that only recently investigators and clinicians alike have become aware that immune mediated conditions go through various evolutionary stages is actually a bit surprising considering that early and late phases in the evolution of the immune response have been known for a long time. In fact, both innate and adaptive immunity are typically time-dependent and utilize entirely diverse cellular and molecular mechanisms (1). Thus, the observation that IMIDs are also time-dependent and rely on different pathogenic mechanisms at diverse times of evolution should not come as a surprise. Immediately after birth the naïve immune system is at the very beginning of being educated and displays a great deal of plasticity to accommodate and acquire the ability of responding to a whole new spectrum of environmentally derived challenges, including the body microbiome, infectious agents, allergens, vaccines, immunizations, dietary antigens, xenobiotics and so on (2). During this early but crucial period of education the immune system must go through crucial steps : the first is to develop *self*

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tolerance, i.e., the ability to distinguish between self and non self (foreign) and establish a controlled state of non-responsiveness (tolerance) to endogenous antigens ; the second step is to selectively develop *exogenous tolerance*, i.e., the ability to recognize and respond to an enormous array of newly encountered environmental antigens without inducing an armful inflammatory response ; finally, the third step is to develop *effective immunity* that allow the elimination or control of infectious, allergic and noxious agents that may cause disease (2).

Conditioning of early and late immune responses

These three steps of immunological education are absolutely essential and carry a "one time only" chance of being implemented correctly as the immune status developed early in life is permanently "imprinted" and maintained into adulthood. The importance of these events cannot be overemphasized because if immune education is incomplete, weak and restricted, the ability to respond and defend the body against a broad range of subsequent inflammatory (or neoplastic) insults may be lost forever. The spectrum of agents and conditions that poses a potential risk of developing IMIDs is incredibly vast and is represented by what is now called the "exposome", the totality of elements that will consistently or predictably challenge the body, including radiation, stress, life style, infections, drugs, diet and pollution (3). Thus, the combination of the type, quantity, timing and recurrence of these factors and the competence of a mature immune system (the "immunome") will determine whether IMIDs will occur or not. The importance of this combination is well exemplified by the temporal evolution of the composition of the gut microbiome (4) and the intimately associated maturation of the mucosal immune system in response to dietary and drug interventions (5). Breast *versus* bottle fed infants develop different enteric microbiota, and even more so if they are exposed to antibiotics early in life. These interventions equate to different immune responsiveness later on and the potential for developing autoimmunity and IMIDs. A recent study shows that the cumulative probability of developing autoantibodies increases in children fed with breast milk without casein hydrolysate (6), and IBD is more common in subjects that received antibiotics during infancy (7). These are just few examples of the life long implications of manipulations of the immune system during critical developmental periods, and how early and late immune reactivity impact on the development of early *versus* late IMIDs. These events are subsequently translated into different pathogenic mechanisms and variable clinical manifestations of IMIDs at different life periods, as shown variations between paediatric and adult IBD. Crohn's disease predominates in paediatric IBD while ulcerative colitis is more common in adult IBD ; in paediatric Crohn's disease there is male predominance compared to adult Crohn's disease ; there is higher ileocolic disease in paediatric compared to adult IBD ; and pancolitis is almost the rule in paediatric but not adult ulcerative colitis (8). It should be remembered that not all that distinguishes early *versu*s late IMIDs is environmentally determined as recent evidence suggests that genetic influences may also influence the appearance of early *versus* late disease (9).

Early and late immune responses in human IMIDs

By definition the tissue damage in IMIDs is mediated by an abnormal or uncontrolled immune responsiveness. Though still relatively limited, accumulating clinical and experimental evidence does support the existence of differences in this responsiveness in early *versus* late disease. In a simple but straightforward study Damen et al. reported that buccal epithelial cells from children with early Crohn's disease produce large amounts of interleukin (IL)-8, but not adults with chronic disease (10). In a more sophisticated study Kugathasan et al. showed a clear distinction between the ability of mucosal T cells to undergo immune modulation by Th1 and Th2 cytokines depending on the stage of their IBD (11). When exposed to the Th1 shifting influence of IL-12 T cells from children recently diagnosed with Crohn's disease or ulcerative colitis produced large amounts of interferon (IFN)-γ ; in contrast, when the T cells derived from children who had the same diseases for at least five years IFN-γ production was no longer upregulated by exposure to IL-12. In addition, children with early but not late Crohn's disease exhibited significantly increased mucosal tissue levels of IL-12p40 and IL-12Rβ2 (11). Taken together, these results indicate that the ability to undergo immune differentiation was present in the early but was lost in the late stages of the IMIDs, providing backing to the notion that distinct immune platforms are behind the clinical evolution of IBD. Additional evidence of immunological diversity in early *versus* late IMIDs can be found in rheumatoid arthritis, which display fairly dramatic differences in synovial fluid cytokine profiles in patients with early *versus* established disease (12). Chronic inflammation often leads to premature senescence of T cells, another sign of immune differentiation accompanying the evolution of IMIDs. Excessive numbers of senescent T cells and the associated telomerase deficiency are well documented in chronic rheumatoid arthritis (13) as well as chronic ulcerative colitis (14), suggesting an erosion of the ability of the adaptive immune system to renew itself in the face of a relentless immune activation. Different pathogenic events have also been described in psoriasis : polymorphonuclear neutrophil infiltrates are found in the dermis in early disease but in the epidermis in late disease ; mast cells are far more abundant in the early than the late stages of disease ; Th17 cells are present almost exclusively in early disease, while Th1 cells are more abundant in late disease, when cytotoxic T cells are also more frequent (15).

Shifts in cellular and soluble mediators underlying early and late immune responses

From the above examples one must conclude that distinct stages of IMIDs are real, and they probably occur in all types of IMIDs. If so, a corollary question is why does this evolution occur, and what prompts it ? For starters, as mentioned above, the normal immune response undergoes a normal and predictable evolution with time, and this evolution is maintained or mimicked during pathological responses. For instance, clear differences in the behaviour of T cells are well documented during an inflammatory process (16). Under homeostatic conditions dendritic cells produce large amounts of TGF-β that induce T regulatory (T reg) cells, which exert a tight control on the number of Th1 and Th2 cells and probably Th17 cells ; in acute inflammation dendritic cells produce less TGF-β but more of IL-6 and IL-23 favouring the expansion of Th17 cells that produce the neutrophil chemoattractant IL-17A, thus explaining the classical neutrophilic infiltration seen in acute inflammation ; as inflammation progresses production of TGF-β, IL-6 and IL-23 is at intermediate levels and T reg cells are less abundant allowing greater expansion of Th1 and Th2 cells whose product cause a relative inhibition of Th17 cells and IL-17A levels. In addition to these endogenous control mechanisms, products from the environment (the exposome) continue to impact on immune cells and their behaviour, as recently shown by the strong modulatory influence of the exogenous substances that bind to the aryl hydrocarbon receptor and may induce a shift in the balance between T reg and Th17 cells (17). Finally, the evolution of the immune response in IMIDS is further reinforced by new data indicating that the traditional one way "rigid" differentiation of naïve T cells into stable Th1, Th2, Th17 or T reg cell subsets may not be so rigid after all ; emerging evidence indicates that such "terminally differentiated" T cells may actually revert or change into other T cell phenotypes, providing a new "flexible" view of T cell function (18). Reflecting logically, if one asks the question of what serves better an effective immune system, rigidity or plasticity, the answer is obvious, as a plastic immune system able to constantly adapt to changing needs dictated by internal and external challenges is clearly the best choice to optimize a response. If so, one could postulate that perhaps in many IMIDs the ability of the immune system to respond in a plastic, specific demand dependent fashion is lost, and rigidity sets in, explaining the chronic stage of disease and the inability to revert to a more acute and more flexible status susceptible to therapeutic immunomodulation.

Novel factor contributing to persistence of IMIDs

In addition to the classical components of the immune system, i.e., T cells, B cells, NK and NK T cells, dendritic cells and macrophages, it is now apparent that practically all other cells types, the so called "non immune" cells, also exert many of the same functions formerly thought to be the exclusive domain of classical immune cells. In fact, from the 1990's to the 2010's, there has been a growing trend toward the study of such non immune cells. Consequently, epithelial, endothelial, and mesenchymal cells as well as platelets and degradation products of the extracellular matrix are being more and more explored in regard to their immune capabilities. This trend is paralleled by the ongoing shift in interest from a dominant T cell centric view of the immune response to one where innate immunity is gaining the upper hand. In reality all classifications in order of importance are completely artificial as adaptive and immune responses always occur concomitantly and influence each other, and all cells in the affected target organ participate in the response. The relevance of this new way of seeing IMID pathogenesis should be obvious. IMIDs are chronic by definition and the resident cells affected by a prolonged immune insult undergo critical changes in function if not in phenotype. On example is the enhanced ability of human intestinal microvascular endothelial cells to bind and retain leukocytes when derived from chronic IBD mucosa (19), a property that persist in successive cell generations regardless of time ; another example is the aggressive phenotype of mesenchymal cells infiltrating chronic rheumatoid arthritis synovium (20). All these cells look like normal cells in both *in vivo* and *in vitro*, but their function is abnormal and contributes to maintain a chronic inflammatory status. Not only do these cells contribute to maintain inflammation, but some of their by-products also do. This is the base of the novel concept of "sterile inflammation", in which inflammation is dependent on the so called damage associated molecular patterns (DAMPs). These include a whole series of products derived from cell death/injury or degradation of the extracellular matrix, such as ATP, RNA, DNA, uric acid, HMGB1, IL-1α, IL-18, heparan sulphate, hyaluronan fragments, etc. All these products are normally hidden from the immune system, but they are freed in an injured tissue microenvironment and incite both a receptor dependent and independent response that results in inflammation (21). All chronically inflamed tissues of IMIDs undergo cell injury or death and matrix degradation, and DAMPs are present in large quantities and contribute to inflammation. In the particular case of IBD, which occurs in a particularly microbe rich organ (the gut), we now have a combination of sterile DAMPs and microbial pathogen associated molecular patterns (PAMPs) which, according to Nathan and Ding (22) epitomize the perfect combination to cause prolonged "non resolving" (late or chronic) inflammation by converging two different and powerful types of immunostimulatory signals.

Evolution of the immune response : implications for immunomodulatory intervention

The last element that needs to be taken into account when discussing early and late IMIDs is time. In humans the best we can do is compare early *versus* late disease in

different groups of patients, but in animal models we can actually follow up the evolution of the IMID from the beginning (early phase) all the way into chronic (late) phase of the disease. These studies have been carried out in experimental model of murine colitis and they have been extremely revealing, as shown by the colitis of IL-10 deficient mice and the ileitis of SAMP1/YitFc mice (23,24). In the first model there is an early typical Th1 response which is reversible by blocking IL-12 or IFN-γ ; this therapy however is no longer effective in the late stages of colitis, when inflammation is now dependent on increased levels of IL-4 and IL-13, indicating a fundamental switch during the disease process from a typical Th1 to a typical Th2 immune response (23). In the second model ileitis is dependent on elevated levels of tumour necrosis factor (TNF)-α and IFN-γ in early disease, while in the chronic phase this Th1 response still persists but it is now accompanied by a late Th2 response mediated by IL-4, IL-5 and IL-13 (24). Thus, at least in these two models of IBD, this form of IMID is unquestionably linked to entirely different immune mechanisms depending on the evolutionary phase of the disease. These remarkable variations in pathophysiology of gut inflammation obviously have therapeutic implications as the molecules to be targeted are clearly distinct in the various stages of the disease process. In humans similar studies are far more difficult to implement, but some evidence also exists that immunomodulation is not equally effective at different stages of the IMID. This is documented in a paediatric study where the response to TNF-α blockade was far more effective and prolonged in children whose Crohn's disease was treated at the very beginning right after diagnosis as compared to the same regimen given to children with chronic disease (25). This suggests that in the future, once the pathogenesis of each IMID is better understood and the various stages of disease evolution are immunologically defined, therapeutic strategies will include choices based on time and not only diagnosis.

Conclusions and prospects

Based on the above discussion and the still limited amount of data on clinical and pathophysiological evolution, the notion of early and late stages of IMIDs seems correct and on reasonably solid ground. So, it seems sensible to propose that IMIDs represent the convergence of primary and secondary factors over a long period of time, during which various phases of the disease occur (Fig. 1). Primary factors determine where the IMID will or not occur and they include the exposome, the genome, the microbiome and the immunome ; once the disease is set in at an early stage (regardless of the presence or severity of the clinical manifestations), then secondary factors and events start impacting on the disease process during the evolution towards the late stage, such as the epigenome, DAMPs, neuroendocrine factors, cell differentiation, angiogenesis, fibrosis, etc. (26). In conclusion,

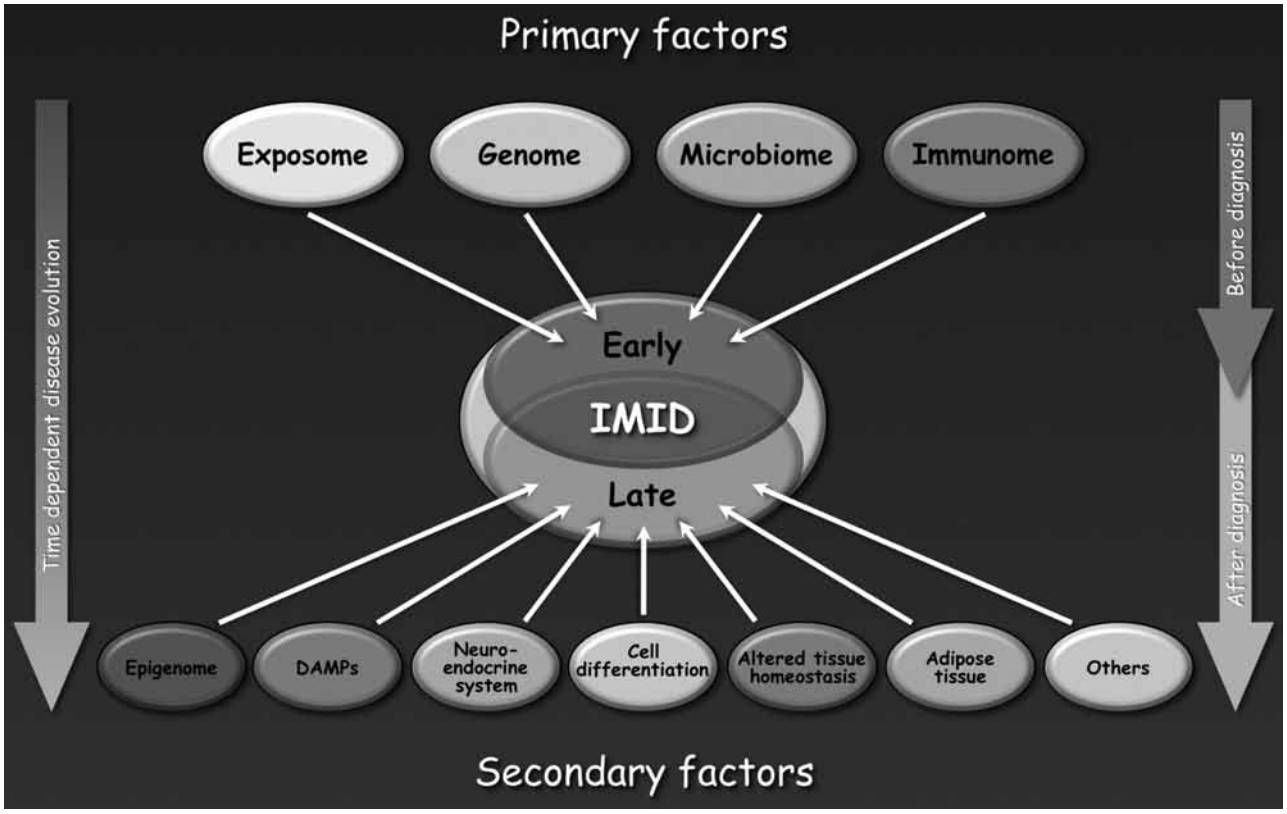

Fig. 1. — Time-dependent convergence of primary and secondary factors involved in the initiation, mediation and progression of IMID pathogenesis from early to late disease (Reproduced with modifications with permission from ref. 26).

because immune pathogenic events do differ in the early and late stages of IMIDs, much more attention should be devoted to the investigation of how an IMID progresses from an early to a late phase, prompting us to think about *when* to study in addition to *what* to study. From a therapeutic perspective early pathogenic events may be more susceptible to immunomodulation and blocking them is conceivably more likely to result in a permanent resolution. Therefore, understanding the differences between the pathogenesis of early and late IMIDs is not only necessary but actually mandatory if the development of better, time-sensitive therapeutic approaches is the ultimate goal.

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