Microbiota and Pro-/Prebiotics


Abstract

The intestinal mucosa possesses a complex epithelial barrier and a well-organized local immune system, which both efficiently protect this internal-external surface against potential microbial aggressions while guaranteeing tolerance towards harmless bacteria or antigens (oral tolerance). There is good experimental evidence that the intestinal microbiota is a main driver for the development of the mucosal immune system. Any perturbations/changes of this interaction with the intestinal microbiota or the microbial colonization process may cause health problems with short- and eventually long-term consequences, such as suspected for allergic or dysimmune disorders. Dendritic cells (DC) play a key role in the initiation of immune responses. Immune responses elicited by intestinal DC differ markedly from those initiated by spleen-derived DC: while intestinal DC induce anti-inflammatory and tolerogenic responses to harmless antigens such as derived from the resident microflora or harmless food allergens, systemic immune activation yields in a strong inflammatory TH1/TH17 reaction to the same antigens. The recent discovery how DC functions are regulated and imprinted by the microenvironment (DC conditioning) will be discussed in this review. High concentrations of retinoic acid or vitamin D metabolites, thymic stromal lymphopoietin and/or transforming growth factor-β (TGF-β) activate signaling programs in DC that yield in priming of regulatory and anti-inflammatory T cell responses. TGF-β is one of the key factors implicated in intestinal immune regulation; it is produced by a large variety of cells in the intestinal mucosa, including intestinal epithelial cells, lymphocytes and monocytes/macrophages/DC. An important anti-inflammatory effect of TGF-β on the immune system is the promotion and generation of FOXP3-positive regulatory T cells in the intestinal compartment. There are first and encouraging data from the treatment of Crohn’s disease, an inflammatory GI condition, that targeted enteral therapy with optimized concentrations of immunoregulatory peptides, such as TGF-β, might of interest for the treatment of inflammatory disorders.

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Introduction

The intestinal mucosa is an internal external surface of over 300 m², exposed to an enormous antigenic load of alimentary and microbial origin. To protect this surface from potentially harmful aggression (pathogens) while guaranteeing tolerance towards harmless antigens (i.e. of alimentary origin or the commensal microbiota), this organ evolved protective strategies, resulting in an efficacious epithelial barrier and highly organized mucosal immune system. The exchange between microflora and intestinal immune system is also an interesting example how a symbiotic interaction is impacting on host health while allowing survival of the microbial mass in the intestine outnumbering by far the host’s own cell number and genetic information. There is good experimental evidence that the intestinal microbiota is a main driver of the development of the mucosal immune system, with a critical phase during initial colonization after birth [1, 2]. This is a tightly controlled and regulated process. Thus, it is easily understandable that perturbations and (quantitative or qualitative) changes of the colonization process may cause health problems with both short- and long-term consequences. In addition, aberrant or pathological function of key molecules indispensable for immune homeostasis within the intestinal mucosa may also result in chronic diseases, such as recently highlighted by the discovery of monogenetic inflammatory gastrointestinal disorders [3–5]. Examples of diseases resulting from a disturbed microbial-host interaction are numerous, such as allergic diseases or dysimmune disorders, particularly those involving the intestinal tract, such as inflammatory bowel diseases. Research over the last years allowed gaining profound insight into the regulation of the immune system, the dialogue with the microbial environment, as well as the potential impact of food-derived antigens on pathological inflammation. These advances were largely stimulated by the introduction of new experimental disease models, such as experimental colitis models, as well as by the discovery of distinct human diseases, highlighting defects in key steps of immune regulation. However, many open questions remain, i.e. the initial triggers causing chronic intestinal inflammation, such as IBD, the cause of food intolerance or the reason for loss of tolerance or lack to acquire tolerance. The present review will focus on the recent knowledge of the regulation of the intestinal immune system with regard to immune-mediated, inflammatory diseases in children as well as treatment strategies based on anti-inflammatory factors, as discussed for transforming growth factor (TGF)-β.
Regulation and Organization of the Intestinal Immune System

Given the important task to protect the intestinal mucosa, while enabling the uptake of large amounts of nutritional products, it is not surprising that the intestine harbors over 70% of immune-competent cells and produces a high amount of immunoglobulins. This reflects its high immunological activity. Indeed, the intestinal mucosa is in a permanent and well-controlled physiological inflammatory state. At its surface, a constant and tightly regulated exchange between host and environment occurs. Privileged sites of interaction are the Peyer patches, such as in the terminal ileum. The intestinal mucosal immune system is differentially organized, and schematically we can separate sites of immune recognition from effector sites. For the recognition of antigenic or foreign structures (of alimentary or microbial origin), macrophages and dendritic cells (DCs) play an extremely important role. These antigen-presenting cells sense microbial and other antigenic structures in the intestinal lumen and initiate immune responses. An immune response elicited by intestinal DCs differs markedly from those initiated by spleen-derived DCs: intestinal DCs induce anti-inflammatory and tolerogenic responses to harmless antigens such as derived from the resident microflora or harmless food antigens, while systemic immune activation causes a strong inflammatory TH1/TH17 reaction. Thus, intestinal DCs efficaciously induce regulatory T cells (Tregs), high levels of IL-10 and more easily Th2 type T cells. This particularity of intestinal mucosal immune responses is not due to different types of DCs (since they all derive from a common pool) but reflects a differing environment (intestinal mucosa vs. spleen or other lymphatic tissues). Recent research clearly confirmed that the functions of DCs are regulated and imprinted by the microenvironment [6, 7]: high concentrations of retinoic acid or vitamin D metabolites, as well as thymic stromal lymphopoietin or TGF-β activate signaling programs in DCs that result in priming of regulatory and anti-inflammatory T cell responses (fig. 1). This interaction between DCs and the microenvironment is highly dynamic, and the process is called DC priming or conditioning. These observations led to approaches to target immune responses on the level of the intestinal mucosa via dietary/nutritional modifications, as well as the modulation of the intestinal flora (such as the use of probiotics). It is interesting to know that naïve T cells, primed by intestinal DCs in the intestinal mucosa or mesenteric lymph nodes acquire specific cell surface markers. These molecules allow T cells, which will circulate throughout the whole body, to get readdressed to the intestinal mucosa, where they migrate to immune effector sides. This process is called homing, and the molecules implicated are specific integrins (i.e. α4β7) and chemokine receptors (i.e. CCR9). The final immune response results
from the balance between effector cells and immunoregulatory cells (fig. 2). As discussed, this is a highly dynamic process largely influenced and modulated by changes of the environment. This requires plasticity allowing the adaptive immune response switching from a tolerogenic to an inflammatory protective response depending on the situation.
As already indicated, TGF-β is one of the key factors implicated in modulating immune responses, together with retinoic acid, many distinct cytokines and chemokines, as well as microbial products [8]. TGF-β is produced by a large variety of cells in the intestinal mucosa, including intestinal epithelial cells, lymphocytes and monocytes/macrophages/DCs. Three distinct isoforms of TGF-β exist (TGF-β1, TGF-β2, TGF-β3) exhibiting similar functional properties. After cleavage of the latency-associated protein which occurs upon homodimerization of two TGF-β molecules, TGF-β becomes biologically active and binds with high affinity to TGF-β receptor II. Upon binding, TGF-β-RI is recruited and subsequently phosphorylated resulting in an active receptor complex subsequently phosphorylating receptor-regulated Smad (RSmad), Smad2 or Smad3. Once phosphorylated, RSmads associate in the cytoplasms with so-called CoSmads (i.e. Smad4) allowing nuclear translocation and binding to specific promoter regions. This signaling cascade is under tight control of inhibitory molecules, such as Smad7, competing with CoSmad, thereby negatively regulating TGF-β signaling.

The effects of TGF-β within the intestinal mucosa are very complex and pleiotropic, since almost all cell types express TGF-β-RII and -RI and are able to respond to TGF-β. Therefore, TGF-β can interfere with the regulation of cell growth, differentiation, survival and apoptosis, extracellular matrix synthesis (fibrogenesis and wound healing). TGF-β exerts modulatory effects on epithelial, endothelial and muscular cells, fibroblasts and most important both positive and negative effects on immune-competent cells. Usually, effects on monocytes and lymphocytes are considered as anti-inflammatory, such as the inhibition of lymphocyte proliferation, cytokine production and differentiation of T helper cells. TGF-β is one of the key factors promoting and generating FOXP3-positive Tregs in the intestinal compartment. An interesting field of research emerged over the last 5 years identifying factors that regulate the balance between effector and Tregs or, in other terms, the balance between pro- and anti-inflammatory immune responses. Various animal models, but also human studies identified a highly plastic and dynamic priming process in the intestinal mucosa, with CD103+ DCs, retinoic acid, TGF-β, IL-6 and several regulatory cytokines, such as IL-23 as key players [9, 10]. Schematically, experimental data indicate that depending on the local gradients of these distinct factors, DCs prime naïve T cells towards TH17 or Treg types. A predominant TH17 development is observed in the presence of proinflammatory cytokines, such as IL-6. However, of utmost importance are the concentrations of TGF-β in this dichotomy between Tregs and TH17: high TGF-β concentrations orientate to-
ward FOXP3 expression and Treg priming, while low concentrations favor generation of TH17 cells. These effects are also an indirect consequence of TGF-β’s suppressive effects on naïve T cell differentiation into either TH1 or TH2 cells. TGF-β was shown to upregulate both transcription factors, Foxp3 and RORγt [9]. It is still unsolved, how TGF-β regulates subsequently the predominant expression of one transcription factor while silencing the other. The presence of other factors potentially affecting cell fate is one additional regulatory mechanism, for instance STAT3-activating cytokines such as IL-21 and IL-23, amplify RORγt induction and Th17 differentiation, while IL-2 was shown to inhibit Th17 cell fate.

Examples of Human Diseases and Use of Nutritional Approaches: the Role of TGF-β

As discussed above, alterations of the compositions of the intestinal microbiota, as well as the function of key regulators of immune homeostasis may lead to chronic inflammatory disorders. One such example is Crohn’s disease (CD), which occurs in genetically susceptible individuals [10]. To date, over 100 susceptibility loci were identified in patients with CD. These genes code primarily for molecules that interact with the microbial environment, such as pattern recognition receptors (most known example: nod2/CARD15), autophagy or molecules implicated in immune regulation of the adaptive immune system (such as IL-23R) or the intestinal epithelial barrier. In patients with CD, a profound dysbalance of the intestinal microbiota is observed (called dysbiosis), which might be a cause or consequence of the chronic inflammatory process [11]. It is now well established that CD is a multifactorial disease with several distinct clinical phenotypes. Treatment approaches are thus quite heterogeneous. The majority of established strategies are based on various anti-inflammatory or immunosuppressive drugs. However, one potent and highly efficacious treatment approach is based on exclusive enteral nutrition (EEN) [12–14]. The efficacy of EEN to induce remission in patients with active CD is as high as steroid medication. There is first experimental evidence that EEN strongly downregulates mucosal inflammatory parameters. In this context, it is interesting to note that one product commonly used, Modulen® IBD, contains high concentrations of naturally occurring TGF-β. It might be of advantage to increase local concentrations of TGF-β during an inflammatory process in the corresponding compartment to obtain an anti-inflammatory effect via targeted enteral nutrition. In addition, some data show that EEN modifies the composition of the intestinal flora. And we now
know that some bacterial strains can also directly impact on the local production of TGF-β, which could result in an additive effect of EEN. These are first and encouraging data highlighting that targeted enteral therapy (in the form of a nutritional approach) might be of interest to patients with an inflammatory intestinal disorder.

In summary, the intestinal mucosal immune system is a highly dynamic and tightly regulated system that adapts to the various challenges it faces every day. External factors, such as vitamins and metabolites, as well as endogenous factors such as growth factors, cytokines and chemokines are potent regulators of immune responses. TGF-β is one of the most important players in the intestinal mucosa allowing switching from inflammatory TH17 responses to regulatory responses indispensable to acquire tolerance. This knowledge might help to design new treatment strategies for immune-mediated bowel disorders.

Disclosure Statement

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References


