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CYTOKINE RESPONSE TO BACTERIAL TRANSLOCATION IN OLD WORLD MONKEYS MODEL OF IBD

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Annotation

The development of inflammatory bowel diseases (IBD), representing various clinical and morphologic forms, is caused by the interaction of genetic, immunologic, and bacterial mechanisms. The cytokine response to bacterial translocation involved in the pathogenesis of the inflammatory process in the intestine was investigated on the model of experimental inflammation in nonhuman *Macaque mulatta* monkeys. Symptoms of inflammation were chemically induced by administration of 5% dextran sulfate. In response to induction, an increase in serum markers of translocation (16S rDNA and sCD14) and stimulation of cytokine response were recorded. The dynamics of cytokine response shows that the duration of DSS-induced inflammation is comparable to the time required for the transition of acute inflammation to chronic inflammation and is characterized predominantly by Th1 cytokine profile.

KEYWORDS: Macaca mulatta, sodium dextran sulfate, microbial translocation, cytokines.

INTRODUCTION

The inflammatory process is controlled by multiple immune regulatory cells as well as pathogenic and commensal microorganisms present in the inflammatory focus. In the gut, populated by a gigantic number of bacteria, controlled inflammation is a crucial imperative to maintain homeostasis, so immune regulatory cells constantly interact with gut bacteria and food molecules, regulating pro-inflammatory effector cells and stimulating anti-inflammatory pathways.[1] In some cases of dysregulation, certain immune conflict reactions develop that induce tissue damage. Events such as destruction of the epithelial barrier, uncontrolled bacterial colonization, unregulated stimulation of immune effectors, and disruption of homeostatic balance may contribute to disease. It should be noted that these phenomena can occur anywhere in the gut.[2,3]

Inflammatory bowel diseases (IBD) are defined as chronic recurrent inflammatory diseases that affect the colon and other parts of the gastrointestinal tract. There are two main types of human RCD: Crohn's disease (CD), which is characterized by transmural ulceration of the small bowel and colon, and ulcerative colitis (UC), in which ulcerative lesions of the colonic mucosa are observed.[4] The global incidence of UC ranges from 24.3 per 100,000 person-years in developed countries to 6.3 per 100,000 person-years in developing countries, while BC ranges from 20.2 to 5.0, with the incidence and prevalence of both diseases increasing. [5]

The microflora that colonize the human intestine are separated from the immune system by a monolayer of closely adhering epithelial cells. Nevertheless, small numbers of bacterial products and even whole bacteria from the intestinal lumen still regularly penetrate this dense epithelial cell barrier, entering the underlying

mucosal tissues, mesenteric lymph nodes, blood or liver in a process called microbial translocation, which is critical for the development and maintenance of immune system competence.[6] Damage to the epithelial barrier is characterized by increased expression of critical cytokines in the blood.[7] The result of a sustained increase in microbial translocation can be chronic systemic immune activation, which inevitably leads to immune exhaustion and loss of immune function.[8]

IBD appear to develop as a result of dysregulation of the homeostatic balance maintained between the gut immune system and resident commensal bacteria.[9] It is important to keep in mind that despite temporary and localized inflammatory effects in the small or large intestine, uncontrolled gut inflammation always has systemic effects on the body.[10] Although the exact etiology of GPC is unknown, it is thought to involve a combination of several factors: genetic predisposition, altered expression of bacterial paternal recognition receptors, alterations in the microbiota, and dietary disturbances. In particular, genomic studies have shown the involvement of more than 160 genetic loci in the emergence of susceptibility to IBD.[11]

Thus, by switching off or disrupting the function of a number of genes associated with IBD (e.g., genes of IL-10, IL-2, STAT3), it is possible to accurately reproduce many of the symptoms, pathology, signaling pathways, and histological features of the disease in rodent models.[12] Moreover, strong evidence for a link between genetic predisposition and loss of microbial tolerance at the onset of chronic colitis has been obtained using examples of genetically susceptible mice that do not develop colitis when kept under aseptic conditions.[13] In addition, chronic inflammatory bowel diseases such as IBD are often associated with previous acute inflammatory disease caused by viruses, bacteria, parasites, dysregulation of the intestinal immune response or autoimmune disorders.[14] Animal modeling can be extremely useful in elucidating the etiology of inflammatory bowel diseases.

The treatment of IBD is determined by the symptoms of the disease and is often lifelong.[15] Current therapeutic strategies for the treatment of IBD include the use of anti-inflammatory drugs, immunosuppressants, antibiotics and biologically active agents. In critical cases or unresponsive patients, therapeutic approaches have to be combined with surgery.[16] However, treatment is not effective in all patients. Some patients develop side effects that complicate the course of the underlying disease. The existing regimens for the treatment of IBD lack specific targeting of the etiologic factor, as well as the ability to stimulate the repair of damaged epithelium, which is important for remission induction.[17] The use of animal models allows the development of new therapies that may induce remission or prevent relapse. Symptoms of IBD can be chemically induced in several species of small laboratory animals, but the monkey model has many obvious advantages, including the close affinity of primate members in terms of biochemical parameters and immunobiological properties.

The results of experimental colitis induced by dextran sodium sulfate (DSS) in monkeys of the species *Macaca mulatta* are presented below. The colitis induced in this way is a rather close model of acute intestinal injury that mimics several types of lesions developing in the wall of the large and (to a lesser extent) small intestine, which is consistent with typical pathology of IBD.[18] This model allows us to investigate the effects on the occurrence and course of the disease besides pharmacologic agents of bacteria and probiotics.

The aim of the study was to investigate the cytokine response to intestinal barrier damage and translocation of bacterial components into the vascular bed using an experimental model of chemically induced inflammatory process in the intestine.

MATERIALS AND METHODS

Animals

Five monkeys of *Macaque mulatta* species were used in the study. All animals come from the colony located in the monkey nursery of IEPaT of the ASA (Sukhum) and were clinically healthy at the time of the beginning of the experiment. The animals were kept and cared for in the nursery in accordance with the existing standards for the care and use of laboratory animals.[19]

Reagents

In the experiment, the sodium salt of sulfated dextran (DSS) with a mol. wt. of 40000 was used as an inducer of acute intestinal inflammation in monkeys.

Experimental design

The initial objective was to determine the effective concentration of DSS. Therefore, the experiment was started with a 3% aqueous solution of DSS, which was allowed to drink *ad libitum* for 5 days. Since no clinical and behavioral signs of poisoning were observed during this time, it was decided to switch to a 5% solution of DSS, which had to be administered *per os* in volumes corresponding to daily consumption rates. Blood samples were collected in the dynamics of the disease (background,1,2,3,6,6,14,16 and 42 days). Blood sera of the samples were frozen and stored at -70^{0}C until the study.

Analysis of inflammatory and translocation markers. After thawing, samples were clarified for 1 min in a microfuge at maximum speed, then diluted 10-fold and the content of IFN-γ, IL-1β, IL-6, TNF-α was determined in duplicates by enzyme-linked immunosorbent assay (ELISA) using Vector-Best kits according to the manufacturer's recommendations. The sensitivity of all used test systems was not more than 1 ± 0.5 pg/mL. Due to the high sCD14 content in blood, samples were diluted

at a ratio of 1:500 and then the concentration was determined using the Hycult Biotech kit. The concentration of 16S rDNA was determined by real-time PC reaction (rt-PCR) with Zymo Res kit.

RESULTS OF THE STUDY AND THEIR DISCUSSION

Increased permeability of the intestinal mucosal barrier leads to translocation of microbial products such as LPS and 16S rDNA, which induce immune activation in the form of an acute inflammatory response. LPS is a glycolipid of the outer cell wall of Gram-negative bacteria that, by binding to membrane CD14 and myeloid differentiation complex (MD-2)-TLR4, activates the nuclear transcription factor NF-κB and the production of IL-6, IL-1β, TNF-α, and type I interferons.[20] To prevent overstimulation of monocytes, the body produces the decoy molecule soluble CD14 (sCD14), which like mCD14 can bind the LPS complex but does not transmit a signal. sCD14 is produced not only by macrophages but also in the liver and is detectable in serum. sCD14 is widely used as a marker of LPS-induced monocyte activation.[21] The concentration of sCD14 in the serum of healthy humans and chimpanzees ranges between 1-3 μg/mL. Although in fact sCD14 is a surrogate marker of microbial translocation, its concentration is a fairly correct predictor of clinical development and outcome of various diseases.[22]

In our studies, we used sCD14 primarily as an indicator of monocyte activation. The concentration of this factor in the blood of intact Macaca mulatta monkeys averaged 1.8 μg/mL. Administration of DSN resulted in a rapid and significant increase in sCD14 content. On some days, levels exceeding 8 μg/mL were recorded. The high concentration of sCD14 in the blood of experimental animals was maintained until the end of the study.

A direct marker of bacterial passage through the mucosal barrier is the bacterial decay product 16S rDNA- 16S rRNA gene of prokaryotes detectable in the circulation. Administration of DSN resulted in a dynamic increase in 16S rDNA content in the vascular bed. On some days, levels above 11 copies/μL were recorded. It should be noted that by the end of the study in 4 monkeys these values decreased and reached baseline values, and in one monkey they sharply increased to 17.84 copies/μL, which is the reason for the average high values of 16SrDNA during these periods.

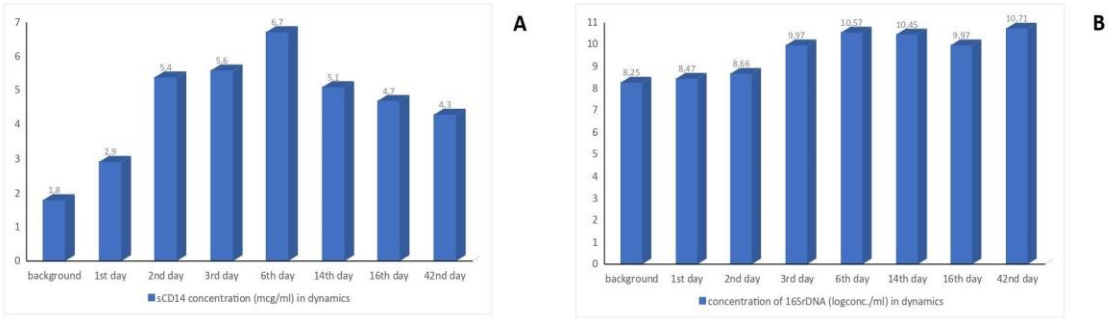

Figure 1: Dynamics of changes in the relative amounts of molecular witnesses of bacterial translocation sCD14 (A) and 16S rDNA (B) in the development of the inflammatory process in Macaca mulatta against the background of oral administration of DSS.

Induction of inflammatory mediators

Cytokines produced by T-helper cells (Th1, Th2 and Th17) have a profound impact on inflammatory processes in the gut. Studies of UC and CD as well as multiple animal models have shown that the pathogenesis of CD is determined by Th1/Th17 cells through the production of IFN-γ and IL-12, while UC by Th2 cells through the production of IL-4,-5,-10,-13. DSS-induced colitis is much closer to human ulcerative colitis, but not to CD, in terms of cytokine profile. Thus, it has been shown that in DSN-induced colitis IL-4 secretion is sharply increased, and deletion of this gene leads to a decrease in the severity of the disease.[23] Proinflammatory cytokines are local proinflammatory mediators produced by macrophages and lymphocytes, as well as epithelial and mesenchial cells, and which are involved in the development of both inflammatory pathogenesis and immunity. These mediators appear to play a critical role in the pathogenesis of IBDs, including ulcerative colitis and Crohn's disease.Increased intestinal permeability with subsequent infiltration of immune effector cells is thought to increase mucosal production of proinflammatory cytokines by both epithelial and immune cells. We studied the production of proinflammatory cytokines IL-6, IL-1β, TNF-α, and IFN-γ in response to DSS exposure. A significant increase in blood levels of all cytokines studied was found. This stimulation is detected very early, on the first/second day after exposure, reaching a maximum on day 6. The expression level of most mediators is significantly higher than the backgroundvalues: TNF-α - 1.5-fold, IFN-γ and IL-1β - 2-fold, IL-6 - almost 3-fold.

After withdrawal of DSS, these mediators remain at high levels, gradually decreasing towards the end of the experiment. This indicates that the duration of inflammation induced by DSS is comparable to the time required for the transition of acute inflammation to chronic inflammation.

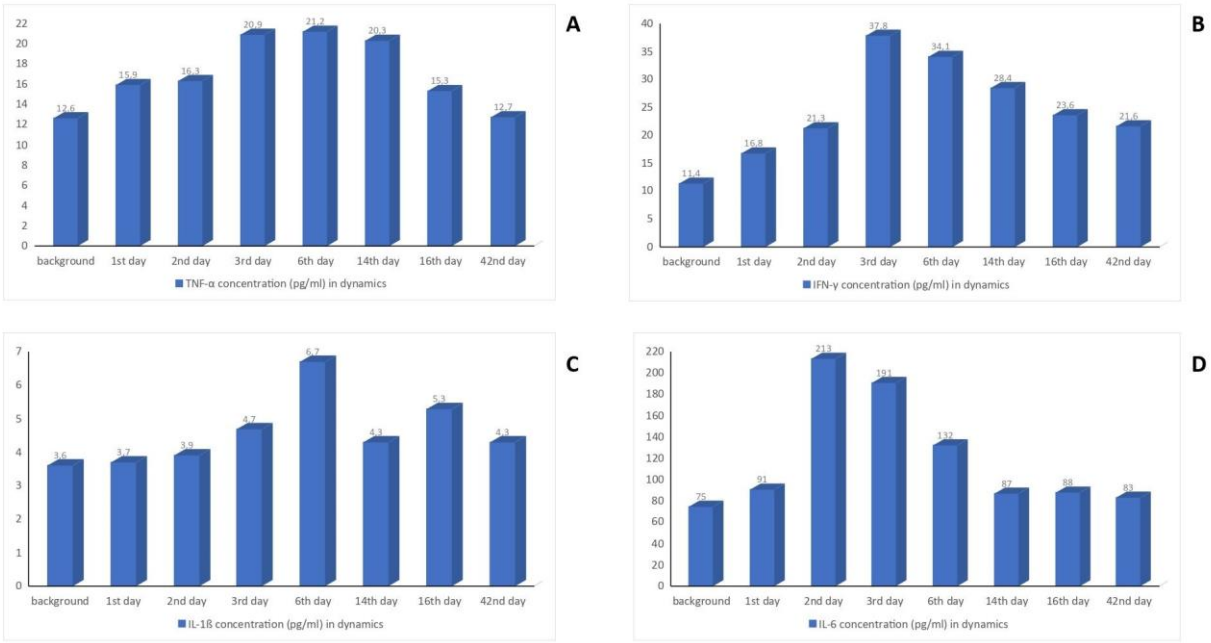

Figure 2: Dynamics of changes in the relative amount of cytokines TNF-α, IFN-γ, IL-1β, IL-6 in the development of the inflammatory process in Macaca mulatta against the background of oral administration of DSS. The level of mediators expression significantly exceeds the background values: TNF-α - 1.5 (A), IFN-γ - 2 (B), IL-1β - 2 (C), IL-6 - 3 times (D).

Cytokines have been shown to exert their biological functions through Janus tyrosine kinase and STAT transcription factors. Experimental IL-6 receptor blockade has shown that this cytokine plays a critical role in the development of Th1-mediated chemically induced colitis by activating the STAT3 signaling pathway.[24] Indeed, STAT3 is the most potent tyrosine phosphorylating enzyme in patients with UC and CD and in mice with DSS-induced colitis.[25] These data suggest that the IL-6/STAT3 signaling pathway plays a critical role in the development and progression of DSS-induced colitis by being a crucial negative regulator.

TNF-α plays a central role in the pathogenesis of Th1 mediated colitis of the CD type and beyond. Thus, expression of TNFR2 receptor and STAT3 transcription activator, through which the signal is transmitted to colonic epithelial cells, increases markedly during the recovery phase of DSS-induced colitis. Cytokine signal transduction through the TNFR1 receptor has a similarly protective effect in mouse models of IBD.[26] A member of the TNFR superfamily, the glucocorticoid-inducible GITR gene is constitutively expressed at high levels on CD4+CD25+ regulatory T cells and at low levels on unstimulated T cells, B cells and macrophages. This receptor in CD4+ T cells is involved in the development and progression of colitis, whereas deletion of GITR protects against chemically induced colitis by suppressing the innate immune response and effector T cell activity.[27]

IL-1β belongs to interleukin (IL)-1 family of cytokines that includes 11 members (7 proinflammatory agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ) and 4 defined or putative antagonist (IL-1R antagonist (IL-1Ra), IL-36Ra, IL-37 and IL-38) having anti-inflammatory activity) Although IL-1 cytokines are found in the bloodstream at relatively low levels, they induce potent inflammatory signals. The biological activity of IL-1 cytokines is controlled at the level of their production and maturation, binding to receptors and post-receptor signaling by natural inhibitors. IL-1β is produced as a biologically inert propeptide that requires cleavage by caspase-1 at its N-terminal region to produce the active form of protein/-converting enzyme (ICE), also known as caspase-1, is an intracellular protease that cleaves IL-1β and IL-18 precursors, thereby forming active cytokines. ICE deficiency accompanied by reduced release of the proinflammatory cytokines IL-1β, IL-18 and IFN-γ provides protection against DSN-induced colitis.[28] Several genetic modifications or mutations associated with dysregulated IL-1 activity and autoinflammatory disorders have been identified in mouse models and patients.

CONCLUSION

The data presented above, obtained on the clinical and experimental model of IBD in lower primates *Macaca mulatta*, testify to the intensification of bacterial translocation after administration of the inflammatory inducer - 5% sodium salt of sulfated dextran (DSS) with a mol. mass of 40000 per os in volumes corresponding to the daily norms of fluid intake (100ml per kg of animal weight). Molecular witnesses of bacterial translocation are 16S rDNA and sCD14, the levels of which increase from the 2nd - 3rd day to a maximum on the 3rd - 14th day of observation, exceeding the background values by a multiple. Immune activation is characterized by early cytokinemia in the form of increased levels of IL-6, IL-1β, TNF-α, IFN-γ as early as 1-2 days after exposure, reaching a maximum on the 6th day. The duration of DSS-induced inflammation is comparable to the time required for the transition of acute inflammation to chronic inflammation.

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