REVIEW

IMMUNOPATHOLOGICAL CHARACTERISTICS OF CRYPTOGLANDULAR AND CROHN’S ANORECTAL FISTULAS

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ABSTRACT
Anorectal fistulas are quite common proctologic disorders. They can be either of cryptoglandular origin or can be associated with Crohn’s disease and chronic ileocolitis. Mechanical obstruction and local infections are prime causes of this pathological condition. Genetic predisposition and inadequate immune response with overproduction of pro-inflammatory cytokines appear prominently in the course of Crohn’s disease. Interferon-γ, a Th1 type cytokine, reflecting the engagement of cellular immune mechanisms, is the first to be produced in the intestinal mucosa. The inflammatory process in the colon mucosa induced by the abundant microbial flora is sustained and turned chronic by the gradual elevation of the local TNF-α and regulatory cytokines levels (interleukin-10, transforming growth factor-β). The number of activated local memory T cells CD4+CD45RBlo increases significantly. The regulatory CD4+CD25+ T lymphocytes producing interleukin-10 increase also trying to counterbalance the cytokine reaction. The chronic inflammatory infiltrates of the colon mucosa are represented by lymphocytes, plasma cells, macrophages. The long-term activation of macrophages by the released interferon-γ leads to tissue damage and potentiation of angiogenesis - a risk factor for carcinoma development. Management of anorectal abscesses and fistulas is complex aiming to alleviate the symptoms, prevent relapses, reduce the risk of sphincter damage and improve quality of life. The main approach (surgery) should be combined with antimicrobial infection control and immunomodulation by intravenous or local administration of anti-TNF-α antibodies.

Key words: anorectal fistulas, Crohn’s disease, cytokines, anti-TNF-α antibodies, immunomodulation

INTRODUCTION
Anorectal fistulas (ARF) are among the most common benign proctologic diseases. Their prevalence as non-specific cryptoglandular ARF is between 8.6 and 18.4 per 100000 people per year, while as associated clinical manifestation they are found in 14-43% of Crohn’s disease patients and in as much as 76% of all patients with inflammatory diseases of the ileocolon. According to the cryptoglandular theory proposed by Prat and Eisehammer, in their former form, ARF are considered a chronic phase in the development of pathologic inflammatory abscesses in the anal glands in humans. Their mechanical obstruction and local infection are main pathogenetic mechanisms in the occurrence and development of ARF. In a similar way they are accepted as consequence of anorectal abscess (ARA), being along with the ulcerations of colon mucosa and strictures the main complications of Crohn’s disease. The clinical manifestations of frequently recurring and fistulizing anorectal disease lead to anal incontinence with poor hygiene and overall decreased quality of life. Nowadays, Crohn’s disease is considered a chronic granulomatous disease of the intestines in which the intestinal wall is thickened and inflammatory altered as a result of the genetic predisposition and development of immune response to infectious agents. The basis of the process is the immune reaction, presenting with overproduction of pro-inflammatory cytokines further deteriorating the intestinal lesions typical for the disease.
ARF are classified by different criteria depending on:
- the extent of anal sphincter involvement: intrasphincteric (submucous and subcutaneous), intersphincteric, transsphincteric (low and high), extrasphincteric and suprasphincteric,
- drainage conditions: open (with active drainage) and closed, respectively complete and incomplete,
- localization of their external communication: external (perianal or open into the abdominal wall) and internal (recto-vaginal, ano-vaginal, recto-vesical and recto-urethral),
- the presence of complications: simple and complicated. Complicated fistulas result from: 1. multiple external fistulous openings (fistula orifices); 2. abscess cavities and branches; 3. proctitis.

Complicated ARF are a common finding in Crohn’s disease, usually high and associated with general manifestations when abscessing. Their exacerbation is a consequence of colitis with diarrhoea, bloody secretion and anal fissures. Patients with simple (non-complicated) ARF show higher frequency of improvement and clinical recovery in comparison with patients with complicated ARF.10

The classical view on the problem is that there is much greater probability of recurrence of ARF in Crohn’s disease due to difficulties in the combined (medication and surgical) treatment.

CYTOKINES AS “CONDUCTORS” OF THE LOCAL IMMUNE REACTION IN ANORECTAL FISTULAS

In ARF combined with Crohn’s disease, the balance between pro-inflammatory cytokines (interleukin-1 [IL-1], interleukin-6 [IL-6], interferon-gamma [IFN-γ], tumor necrosis factor-alpha [TNF-α]) and regulatory cytokines (interleukin-10 [IL-10], transforming growth factor-beta [TGF-β]), is essential for maintaining the local immunity of the intestinal mucosa.11 Cytokines are produced from various cells. IFN-γ is synthesized by Th1 lymphocytes, conducting cell-mediated immunity and affecting activation of macrophages. Its production is dependent on TNF-α levels.12,13

In ARA and ARF that are concomitant complications of Crohn’s disease or other forms of chronic colitis, the local overproduction of pro-inflammatory cytokines IFN-γ and TNF-α along with IL-10 and IL-12, is main pathogenetic factor that sustains the inflammatory process in the intestinal mucosa. The abundant microbial flora in the terminal ileum and the colon is a powerful inducing factor for cytokine synthesis. Studies of experimental mice models show that IFN-γ is the first and the most rapidly increasing cytokine preceding the inflammatory cellular alterations in the intestinal mucosa and the activation of local lymphocytes.4 Following the changes in IFN-γ and correlating with the severity of inflammation, the local levels of TNF-α, IL-4, IL-5 and IL-10 are raised. The last two cytokines possess modulating effect on later stages of inflammation. Increased local levels of IFN-γ may serve as a prognostic marker of recurrence of the disease. (Fig. 1)

Cytokine concentrations are measured in in vitro five-day cell culture supernatant of colon mucosa biopsies acquired by colonoscopy.14 Enzyme-linked immunosorbent assay (ELISA) is used. Another possibility is immunohistochemical determination of cytokine levels in mononuclear cells from lamina propria.15

Our long-term clinical and scientific studies in patients with various inflammatory and infectious processes (gastrointestinal forms of salmonellosis, viral hepatitis B, Mediterranean spotted fever) convincingly show that the determination of serum and local levels of some pro-inflammatory cytokines (IL-1, IL-6, IFN-γ TNF-α) has diagnostic and prognostic significance for the development of the disease.16-19 We therefore decided that the determination of local cytokine levels from intestinal biopsy material in patients with ARF may be used for assessment of inflammatory reaction development.

OTHER IMMUNOLOGICAL FACTORS TRIGGERING ANORECTAL FISTULAS

The role of T-lymphocytes in Crohn’s disease as immune cells in the development of chronic inflammatory diseases of the colon, including complicated ARF, is demonstrated by experiments on mice with chronic ileitis and perianal fistulas.4 Flow cytometric analysis of lymphocytes from mesenteric lymph nodes of laboratory animals shows decrease in CD4+ T-lymphocytes with naive phenotype (CD4+CD45RBhi population), while the percentage of activated memory cells CD4+CD45RBlo, as well as regulatory CD4+CD25+ T-lymphocytes (T regs), expressing alpha-chain of IL-2, is significantly greater. The increase of cells with activation markers and of T regs with disease progression is a sign of occurrence of memory and activated T-cells. Activated T regs in intestines play an important role in
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The control of cytokine imbalance. Isolated from intestinal mucosa of animals with inflammatory diseases of the colon, they are considered intestinal inflammation regulators and responsible for induction of immune tolerance in the colon mucosa via IL-10 production.

Continuous inflammatory process in ARA and ARF associated with certain microbial agents is a major factor of immune suppression in patients. Abscess etiology is polymicrobial, being associated in over 70% of the cases with obligate (Bacteroides fragilis, Peptostreptococcus spp., Prevotella spp., Fusobacterium spp. etc.) and facultative anaerobic (S. aureus, Streptococcus spp., Escherichia coli) bacteria. Brook and Frazier report the highest participation of Gram positive bacteria (staphylococci and streptococci), followed by E. coli among facultative anaerobes. Our previous studies show that E. coli and other gram negative bacteria are isolated in more than half of the patients with abscess forms of ARF, and gram positive bacteria (enterococci, staphyloococci) account for one third of the isolated facultative anaerobes. We consider the high percentage of E. coli isolated from the investigated purulent material to be a disturbing fact since, according to Hamalainen and Sainio, these cases are prone to chronicization with fistulization of the process. Risk of sepsis development is present in considerable share of the patients. Based on the hypothesis that prolonged inflammation caused by infectious agents leads to immunosuppression, our investigations of peripheral blood from ARF patients prove functional insufficiency of phagocytic reaction with imbalance in NBT test, total leucocyte count and neutrophil percentage. Humoral factors of systemic acquired immunity (serum immunoglobulins A, G and M) are however scarcely engaged in the abovementioned inflammatory processes, which implies the predominant role of local immune response.

On the other hand, the overactivation of the immune system in Crohn’s disease is related to the occurrence of autoimmune or allergic reactions. In 13 to 100% of the patients with Crohn’s disease, including patients with ARF, perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) are

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**Figure 1.** Intercellular interactions through cytokines in the intestinal mucosa in anorectal fistulas with Crohn’s disease.

The contact of antigen presenting cells (APC) - dendritic cells and macrophages, with microbial antigens leads to activation of intestinal T lymphocytes in Peyer’s patches and lamina propria after stimulation with IL-1. One of the Th-helper subpopulations - Th1, produces IL-2 for proliferation and differentiation of T and B cells. Th1 also release IFN-γ, stimulating the activation of intestinal macrophages which begin to synthesize the pro-inflammatory cytokines TNF-α and IL-1 with cytotoxic effect, blood vessels alterations and fever. IL-2 also stimulates other T cells - Th2, which synthesize IL-4, helping the differentiation of B lymphocytes to plasma cells which in turn produce secretory IgA. T-regulatory cells (T reg) play controlling role over the inflammatory process in the intestinal mucosa. They produce regulatory TGF-β and IL-10. TGF-β supports the differentiation of macrophages, the secretion of extracellular matrix proteins (EMP), wound healing and tissue fibrosis. IL-10 inhibits the cell released IFN-γ and neutralizes the inflammatory effect. A small part of cells involved in the inflammatory reaction are cytotoxic T lymphocytes (Tc), which, activated by IL-2, kill the microorganism-infected target cells.
found. Allergy to cow milk proteins is considered to be one of the reasons for ARF and anal fissures development in Crohn’s disease, since it leads to chronic obstruction and proctitis.

**LOCAL IMMUNOHISTOLOGICAL ALTERATIONS IN COLON MUCOSA IN ANORECTAL FISTULAS**

The morphological histological alterations in the colon mucosa of patients with Crohn’s disease also provide evidence of involvement of immune mechanisms in the development of ARA and ARF as complications of inflammatory diseases of the colon. In these cases the inflammatory reaction is presented by mononuclear and polymorphonuclear cells in lamina propria and neutrophils in the epithelium. Granulomatous alterations with polymorphonuclear giant cells in the colon mucosa - a sign of chronic inflammation, may be found in patients with Crohn’s disease. Except for macrophages, lymphocytes and plasma cells also infiltrate the intestinal mucosa. Prolonged activation of macrophages leads to tissue damage due to the released by them substances, one of which - nitric oxide (NO), directly damages DNA, inhibits DNA reparative enzymes, suppresses caspase activity and apoptosis, and stimulates angiogenesis. The inflammatory process generates pro-inflammatory cytokines and prostaglandins, which also potentiate angiogenesis. Cancerogenic products like reactive oxygen species are produced at the site of the chronic inflammatory reaction. That is the reason for increased risk of carcinoma in case of prolonged inflammatory benign alterations in the intestinal mucosa in ARA and ARF, as well as in anal fissures. These benign anorectal diseases also enhance the negative role of human papillomavirus (HPV) in cancerogenesis initiation facilitating its access to the anal epithelial cells.

In allergy-associated chronic obstruction in patients with Crohn’s disease the immunohistologic alterations of the rectal mucosa are presented with cubic or plasmatic metaplasia of the epithelium, infiltration with plasma cells and lymphocytes and presence of lymphoid nodules in lamina propria.

The findings of local immune alterations in patients with ARF are supported convincingly by experiments with mice with chronic ileitis and fistulization. The ulcerations of anal mucosa, the fissures near the anus and ARF in the mice are accompanied by occurrence of chronic inflammatory infiltrates of lymphocytes, plasma cells and macrophages in the anus and the rectum, as well as by accumulation of neutrophils in the perianal soft tissues. Loss of normal villous architecture of the intestinal mucosa and thickening (to the extent of hypertrophy) of the muscle layer of the intestinal wall from the distal to the proximal compartment of the ileum are also observed.

**NOVEL COMBINED TREATMENT (SURGERY AND IMMUNOMODULATION) OF ANORECTAL FISTULAS IN CROHN’S DISEASE**

The modern therapy used for ARFs, especially the type presenting as complications of Crohn’s disease or chronic inflammatory diseases of the colon, is still a challenge to surgeons and gastroenterologists, because full recovery and prevention of disease recurrence are hard to achieve while keeping a better quality of life. There are two main approaches in the treatment of ARF in Crohn’s disease: surgery and conservative treatment (Table 1). The current trends demand definitively a combined therapy using both approaches.

Surgery is the classical therapeutic approach in treating ARA and ARF in Crohn’s disease. The goals in this approach are symptom alleviation, ARF-associated draining of ARA, reduction of disease recurrence and danger of damaging the anal sphincters by preventing the development of anal incontinence, even to the point of avoiding the need for proctectomy in some rare cases. Currently, surgical interventions in this field include: opening of the fistula tract by one- or two-stage fistulotomy (in low ARF); seton treatment with non-cutting (draining) ligatures (in higher fistulas with involvement of considerable part of the external anal sphincter), or plastic covering of the internal fistulous opening with endorectal forward-shifted flap (as an alternative to the two classical methods but possible only in patients without macroscopic signs for rectal inflammation). In ARF, complicated with Crohn’s disease, a trend for less aggressive surgical approaches, including drainage by insertion of non-cutting setons, is promoted, since more aggressive surgical procedures increase the risk of anal incontinence. For these complicated cases we agree with most of the authors and prefer to use the most novel surgical techniques for obturation of ARF by instilling fibrin glue into them to attach them, or by occlusion of the lumen by implantation of biological anal fistula plugs, manufactured from porcine small intestinal submucosa - Surgisis®-Anal Fistula Plug. The surgical treatment we have been performing for several years in patients with chronic ARF, including instilling of fibrin glues, is safe, easy to perform and effective in more
than 70% of the patients, preventing development of anal incontinence and the need of additional therapy.35,36

Drug therapy in perianal fistulas in Crohn’s disease includes: 1. standard medications, including immunomodulators; 2. hyperbaric oxygen therapy and 3. anti-TNF-α antibodies.

The drug typically used here are: oral 5-aminosalycilates; antimicrobial drugs (metronidazole, ciprofloxacin); corticosteroids for systemic and topical application (prednisone and beclometasone dipropionate), and immunomodulators (azathioprine, cyclosporine, 6-mercaptopurine, methotrexate, tacrolimus).37 Despite their application, the relapses of ARF are quite frequent and the fistulas are difficult to heal. Furthermore, the immunomodulators applied in these cases have serious side effects such as pancreatitis, hepatitis and severe leukopenias. Hyperbaric oxygen therapy is still not widely accepted alternative method. It is applied in patients with severe perianal complications of Crohn’s disease. The therapy’s mechanism of action is growth suppression of anaerobic microorganisms which in addition to inducing local inflammation leads to ARA with development of ARF. It is carried out in series of hyperbaric pressure of 2.5 atm for 20 to 90 min, accompanied by local administration of 100% oxygen.38

The interest in investigating the cytokine imbalance in the intestinal mucosa in inflammatory intestinal diseases does not subside due to the appealing possibility that pro-inflammatory mediators are neutralized through proper therapy. Therapeutical approaches are focused on application of anti-inflammatory molecules. The need for such an approach is determined by the fact that complicated ARA and ARF in Crohn’s disease may not be always successfully treated with surgical interventions because of risk of anal incontinence.

Candidates in the modern strategy for modulation of the inadequate immune response in Crohn’s disease are two biological drugs: 1. humanized chimeric antibody against TNF-α (infliximab) and 2. human anti-TNF-α antibody (adalimumab).39,40

The application of TNF-antagonists from the mid-90’s of the last century is a novelty in the treatment of Crohn’s disease and ulcerative colitis, especially in patients who are resistant to therapy with conventional medications like corticosteroids and immunomodulators.

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<th>Approach</th>
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<td>Surgical</td>
<td>Fistulotomy</td>
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<td>Seton with drainage ligature</td>
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<td>A plastic cover of the internal fistula opening with endorectal flap</td>
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<td>Drainage by inserting non-cutting setons</td>
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<td>Obturation by instilling fibrin glue or by occlusion of the lumen by implantation of biological anal fistula plugs</td>
<td>In complicated ARF with Crohn’s disease</td>
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<td>Medications</td>
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<td>Anti-TNF-α antibodies</td>
<td>In patients, resistant to standard therapy; neutralizes TNF-α and lowers IFN-γ</td>
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Infliximab has local mechanism of action on the intestinal mucosa, related with neutralization of TNF-α. The antibody suppresses also the quantity of T-cells, producing TNF-α cytokine in lamina propria by stimulation of their apoptosis and reduction of IFN-γ production by intestinal T-cells. The reduced cytokine production after the treatment correlates with clinical improvement in about half of the patients - remission of the disease or even healing of the fistulas.

Treatment with Infliximab in Crohn’s disease is conducted either by intravenous infusions or by local injections. Venous infusions are three times daily in a dose of 5 mg/kg for 2 hours, at certain intervals - most often at 0, 2 and 6 weeks. Application of prolonged infusions (for more than 40 weeks) is possible. In case of systemic therapy with infliximab the following side effects may be observed:

- autoimmune reactions - appearance of antinuclear antibodies or antibodies against infliximab in 7-10% of patients;
- anaphylactic and delayed type hypersensitivity reactions in repeated application in 20% of patients;
- infectious complications - reactivation of tuberculosis and pulmonary infections;
- appearance of carcinomas or lymphomas.

Local injections are applied in the treatment of non-complicated ARF in Crohn’s disease. Infliximab in a dose of 20 mg dissolved in 10 ml saline is injected in the primary fistula tract and around the external fistulous opening. The procedure is preceded from application of anesthetic drug (5 ml lidocain in 3 ml 8.4% bicarbonate solution). Three to five injections of infliximab are applied each week until the secretion entirely discontinues.

The effect is considered positive if reduction or total cease of fistula secretion for at least 4 weeks is achieved. External perineal fistulas are better treated by topical application in comparison to the internal (recto-vaginal and entero-vesical) ones.

The local treatment with infliximab solution is believed to prevent the side-effects observed in systemic infusions.

Adalimumab is applied in dosage of 80 or 160 mg weekly at the beginning of the therapy and 40 or 80 mg after the second week. It is followed by supportive treatment with 40 mg every second week. Studies of patients with fistulizing Crohn’s disease show good effect of the medication in achieving remission.

Generally, TNF antagonists stimulate ARF healing, increase the effectiveness of surgical intervention and reduce the risks associated with it.

CONCLUSIONS

Anorectal abscesses and fistulas are frequent complications of cryptoglandular disease and Crohn’s disease or chronic inflammatory bowel disease. The etiology and pathogenesis of their occurrence and development, as well as of the diseases associated with them, are not quite clear. The prolonged inflammation of the colon wall causes local hyperproduction of pro-inflammatory and regulatory cytokines. The intestinal inflammation is controlled locally by a special population of regulatory T-cells. By presenting the main imunno-pathogenic mechanisms playing important role in the development of the inflammatory process in these diseases, we would like to underline the necessity of complex therapeutic approach in ARF, especially for those associated with Crohn’s disease. This approach is concurrent with the modern trends for combined therapy - surgical treatment, antimicrobial control of infection and immunomodulation. Most of the research show rather encouraging results in using anti-TNF-α antibodies.

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ИММУНОПАТОЛОГИЧЕСКИЕ ОСОБЕННОСТИ АНОРЕКТАЛЬНЫХ ФИСТУЛ КРИПТОГЛАНДУЛЯРНОГО ПРОИСХОЖДЕНИЯ И ФИСТУЛ ПРИ КРОНА БОЛЕЗНИ

Б. Хаджиеv, М. Мурджева

РЕЗЮМЕ

Аноректальные фистулы представляют собой один из самых часто встречающихся проктологических заболеваний. Это фистулы крипто-гландулярного происхождения или фистулы, связанные с болезнью Крона-а и с хроническим илеоколитом. Ведущим механизмом в их развитии является механическая обструкция и локальная инфекция. При Крона болезни наследственная предрасположенность и появление неадекватного иммунного ответа со сверхпродукцией проинфилтратом цитокинов являются основными моментами в ходе заболевания. В кишечной слизистой оболочке раньше всего проявляется интерферон-гамма Th1 тип цитокин, маркирующий инфилтрацию клеточных иммунных механизмов. Постепенное повышение локальных уровней опухоль-некроз фактора-альфа и регуляторных цитокинов (интерлейкин 10, трансформирующий ростковый фактор-бета) приводит к поддержанию и хронификации воспалительного процесса в слизистой толстой кишки, индуцированного бактериальной флорой. Значительно увеличивается относительная часть локальных активированных T клеток, память CD4+CD45RBlO. Повышается и число регуляторных CD4+CD25+ стволовых клеток, продуцирующих интерлейкин 10, пытаясь контролировать дисбаланс в цитокиновой реакции. Хронические воспалительные инфильтраты в слизистой оболочке толстой кишки, представленные лимфоцитами, плазматическими клетками, моноцитами. Продолжительное активирование макрофагов отделенным интерфероном-гамма приводит к повышению уровня интерлейкина-10, что приводит к улучшению состояния и уменьшению воспалительных процессов в кишечнике. Лечение аноректальных абсцессов и фистул комплексно и ставит себе целью облегчить симптомы, предотвратить рецидивы и улучшить качество жизни. Ведущим подходом является комплексное лечение с помощью антивоспалительных средств, антибиотиков и других препаратов.