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Immunology 4323
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Group #1

Causes, Methods of Treatment, and Current Research on Crohn's Disease

Etiology - Jose

While the cause for Crohn's disease is not known, there are a number of factors that can lead to development. Factors such as heredity and malfunctioning immune system can play a role. While the immune system is supposed to help combat pathogens, in some instances an abnormal immune response can cause the immune system to attack the cells in the digestive tract as well. In addition, individuals with a family history of Crohn's disease are more likely to have this disease. Finally, there are a number of risk factors for Crohn's disease such as age, ethnicity, cigarette smoking, nonsteroidal anti-inflammatory medications, and environmental factors.

Complications of Crohn's disease can include bowel obstruction, ulcers, fistulas, anal fissures, malnutrition, and colon cancer. In addition, Crohn's disease medication can cause problems in the immune system which can lead to developing lymphoma skin cancers and increased risk of infection.

Pathogenesis –Jose

In Crohn's disease, the body's immune system begins attacking healthy cells in the GI tract, causing inflammation. This causes a defect in the mucosal barrier in the intestine which leads to an uptake of luminal antigens, which results in mucosal inflammation caused by APC cells. As a result, T helper cells such as Type 1 T-helper lymphocyte (T_H1) and T_H17-related cytokines get activated. Once T helper-cells become activated by APC's, they release large amounts of pro-inflammatory cytokines. In addition, the selective expression of vascular adhesion molecules provides a mechanism for targeted recruitment of T-cell subsets to the small intestine rather than the colon. Inflammatory cytokines such as TNF, IL-1β and IL-6 upregulate local endothelial expression of vascular cell adhesion molecule 1 (VCAM1), very late antigen 4 (VLA4), and ICAM1, that cause circulating neutrophils and monocytes to adhere to the inflamed endothelium. In summary, in Crohn's disease, inflammation is initiated by antigens normally present in the intestines and results in an uncontrolled inflammatory response mediated by activated T helper cells and pro-inflammatory cytokines such as TNF alpha.

Effect of Peyer's patches – Huson

Peyer's patches are the site of many B cell follicles and T cell areas and are most common in the ileum of the small intestine. They are assumed to be the area of origin of inflammation leading to Crohn's disease. A single layer of epithelial cells, follicle associated epithelial cells (FAE), covers each of the follicles in the Peyer's patches and are more accessible to antigens. Erosion of these lymphoid follicles may cause the initial inflammation leading to the disease. When bacteria bind to intestinal epithelial cells, it leads to infection and production of cytokines. Then the inflammatory response recruits dendritic cells to the site of inflammation. Researchers have hypothesized that a defect in the barrier of FAE cells allows an increased permeability to bacteria in comparison to protein antigens which initiates the inflammatory response in the lumen

that is characteristic of Crohn's disease. The small inflammations occurring at aggregates of lymphoid follicles, if untreated, it eventually becomes ulcers. There is a correlation of the occurrence of ileal Crohn's disease in 20 to 30 year-olds and an increase in the number of Peyer's patches at that age. Symptoms of Crohn's disease are seen about 5 years later, which is enough for chronic infection to occur.

Effects of Neutrophil Migration - Amy

Neutrophils are the first immune cells recruited to the site of inflammation, and their action is crucial to limit invasion by microorganisms. In addition, they play an essential role in proper resolution of inflammation. When these processes are not tightly regulated, they can trigger positive feedback amplification loops that promote neutrophil activation, leading to significant tissue damage and evolution towards chronic disease. Defective chemotaxis, as observed in CD, can contribute to the disease through impaired microbe elimination.

In normal conditions, the single-layered intestinal epithelium constitutes a physical and immunological barrier that prevents contact between luminal microbiota and intestinal mucosa. When the epithelium is damaged, neutrophils are crucial to protect the body from invading pathogens. Under physiological conditions, neutrophils are cleared from the circulation in the liver, spleen, and bone marrow. When not properly eliminated, neutrophils can contribute to significant tissue damages during acute and chronic diseases. This destructive potential of neutrophils requires a tight control of their action into tissues.

Studies have shown that a reduction in the number of neutrophils migrating to the sites of skin abrasions or intestinal biopsies in patients with CD. These leukocytes constitute the first line of defense after microbes and organic debris breach the mucosal barrier. A delay in their accumulation can lead to an abnormal level of persistence of exogenous material within the bowel wall. Subsequent uptake and restriction by macrophages could then produce the granulomata characteristic of CD.

Using skin window chambers reveal a defect in neutrophil recruitment in CD patients. In vitro tests show that neutrophils themselves are competent and that the decreased accumulation of these cells can be attributed to the presence of circulating inhibitors of chemotaxis in patient serum or to an inappropriate release of chemotactic mediators by resident macrophages. Reduced recruitment of neutrophils to sites of pathogen invasion causes persistence of bacteria into tissues and possibly within macrophages. Inefficient clearance of bacteria by macrophages drives the formation of granulomas found in CD, leading to an upregulated adaptive immune response. Generally, extravasation of neutrophils from the vasculature to inflamed tissue follows the following steps: tethering, rolling, adhesion, crawling, and then transmigration. Stimulating the immune system may calm the subsequent excessive adaptive response. Defective neutrophil recruitment resulting from alterations in macrophage function can be counteracted by GM-CSF administration.

Granulocyte macrophage - colony stimulating factor (GM-CSF) and granulocyte - colony stimulating factor (G-CSF) are used to correct the defect in neutrophil recruitment. Although G-CSF is used to increase systemic neutrophil numbers, little is known about its effects on their recruitment into the tissues. Cantharidin blisters have been used to examine the accumulation of leukocytes and inflammatory mediators in newly created inflammatory lesions in subjects with IB disease, and to assess whether systemic G-CSF modulates the response in CD. Since neutrophils and macrophages are non-organ specific, any cellular defect predisposing to CD is likely to be present at extraintestinal sites, such that investigations may reveal abnormalities of pathogenic

relevance in the bowel. It is shown that neutrophil migration to sites of rectal or ileal trauma in a model analogous to the skin window was reduced in CD. Fewer neutrophils were recruited into blisters by 24 hours, confirming the findings of previous studies that used skin windows. This was observed in patients with quiescent disease on no medications. In concordance with impaired neutrophil accumulation, production of secreted neutrophil chemoattractants was diminished in CD.

Current Statistics - Huson

According to the Centers for Disease Control and Prevention (CDC), there are about 26 to 199 cases of Crohn's disease per 100,000 people. The exact number is impossible to determine because the CDC does not have standard criteria to diagnose the disease, and it is often misdiagnosed as a different condition. The disease has become more prevalent in the U.S. than anywhere else in the world at an estimation of half a million people. It is unknown why the disease has increased so significantly in the United States. Crohn's disease is most likely to develop in individuals between the ages of 20 and 29, smokers, and those who have a family history of the disease. Although the mortality rate is slightly higher in individuals with Crohn's disease due to confounding effects with other diseases or conditions such as cancer, death attributed specifically to Crohn's disease is uncommon.

Medications and their Effects on CD

Effect of Non Steroidal Anti-Inflammatory Drugs such as Celebrex - Amy

Non Steroidal Anti-Inflammatory Drugs (NSAIDS) are one of the most commonly used medications for the treatment of inflammatory conditions. However, high doses of NSAIDs are shown to be related to gastrointestinal toxicity, including mucosal injury, upper GI, small bowel, or colonic bleeding. Pathogenesis of Inflammatory Bowel Diseases (IBD), particularly Crohn's disease (CD), includes environmental factors, genetic background, host intestinal flora, and the immune system of the host. There have been several mechanisms responsible for NSAID induced GI toxicity. These include increased mucosal permeability, NSAID induced intracellular ATP deficiency, increased enterohepatic circulation, and prostaglandin synthesis. Prostaglandins play an important role in mucosal defense. NSAIDS may initiate IBD, or cause reactivation of quiescent disease and induce GI complications.

NSAIDs work by stopping an enzyme that stimulates changes in the body from performing its function. This enzyme is known as cyclooxygenase (COX). COX has two forms, COX - 1 and COX - 2. COX- 1 aids the kidneys and guards the stomach lining against digestive chemicals. COX - 2 makes prostaglandins, which are hormone-like substances that cause inflammation, pain, and fever. NSAIDS relieve inflammation by blocking the activity of both COX 1 and 2. This might result in abdominal pain, and sometimes even ulcers in the stomach and/or duodenum, as well as bleeding.

COX - 2 inhibitors were created to counter these effects. COX -2 inhibitors include drugs such as celecoxib and etoricoxib. COX -2 inhibitors block the action of the COX - 2 enzyme and therefore stop pain and inflammation. Since COX -1 isn't being affected, the common GI symptoms of NSAIDs are not provoked. Patients can use COX - 2 inhibitors for brief periods of time without risking flare ups of their disease symptoms. New studies show that although COX - 2 inhibitors were previously thought to cause relapse in symptoms of CD, they actually significantly reduce the likelihood of symptom recurrence. These studies are not consistent however, and there is uncertainty in these results.

Effect of Anti-inflammatory Medications such as Sulfasalazine (SSZ) - Tai-Feng Wu

Sulfasalazine (SSZ) is well known as a disease modifying antirheumatic drug (DMARD). As a DMARD, SSZ is used to treat rheumatoid arthritis and certain autoimmune diseases. It not only can relieve the swelling, the pain and the stiffness of inflammatory arthritis, but can also prevent joint damage. SSZ can also be used to treat inflammatory bowel diseases, such as Crohn's Disease. The structure of SSZ is composed of 5-aminosalicylic acid (5-ASA) and sulfapyridine linked by an azo bond. While sulfapyridine is the main source of the side effects, the 5-ASA is the main source of the benefit for patients. The mechanism of the 5-ASA can breakdown to five contributions: Inhibition of cytokine synthesis, Inhibition of prostaglandin and leukotriene synthesis, Free radical scavenging, Immunosuppressive activity, and Impairment of white cell adhesion and function.

The effects of two of the five contributors are explained: Inhibition of cytokine synthesis and Immunosuppressive activity. First, the main function of 5-ACA is anti-inflammatory. Since peroxisome proliferator activated receptor-gamma (PPAR-gamma) is downregulated in patients with inflammatory bowel disease, 5-ACA can induce PPAR-gamma gene expression to suppress the activation of cytokine NFkB and toll-like receptors (TLRs). On the other hand, active inflammatory bowel disease is found on enhancing mucosal production of proinflammatory cytokines. The biologic functions of the proinflammatory cytokines interleukin 1 (IL-1), tumor necrosis factor alpha (TNFa), IL-2, IL-8, and NF kB can also be inhibited by 5-ASA. Secondly, SSZ and 5-ASA are found to have immunosuppressive activity. The synthesis of lymphocyte DNA and cell cycle are blocked by SSZ and 5-ASA outside of the body to prevent pathogenic T-cell and B-cell populations to grow. Also, because 5-ASA can prevent the early T-Cell activation gene to accumulate for IL-2, it can inhibit the proliferation, subsequent activation, and differentiation of T-cell.

Effects of Immunosuppressive Drugs such as Methotrexate – Christie

Azathioprine (AZA) and 6-mercaptopurine are the most commonly used immunomodulators, but research into alternatives has given patients who cannot tolerate these drugs some relief. One alternative, called methotrexate (MTX), binds to dihydrofolate reductase and inhibits DNA synthesis, resulting in an anti-mitotic action. It's anti-inflammatory action results in an increase of adenosine, which decreases pro-inflammatory cytokine production. Its effect takes 8-12 weeks to develop, and more than 40% IBD patients experience side effects such as liver and gastrointestinal toxicity, infections, and myelotoxicity. These effects can be decreased with folate supplementation. In placebo-controlled trials for Crohn's disease (CD), MTX proved to be effective for the induction and maintenance of clinical remission and to reduce the use of corticosteroid. In this study, MTX was administered to 341 patients (262 CD; 79 UC) out of 2,660 patients. Its effectiveness was found to be 59.5% in CD and 40% in UC. Patients on MTX therapy obtained remission in 87.4% and 69.2% for CD and UC patients respectively. The increase of adverse events occurring caused treatment discontinuation in 39.4% of all cases.

Limited data of the effectiveness of MTX in clinical practice is available, as it is mainly used in rheumatic inflammatory disorders rather than IBD. Overall, MTX has been administered to only 2.1% of patients, despite its effective rates, 64.1% in CD patients and 47.8% in ulcerative colitis (UC) patients. Likely reasons for its limited use pertain to concerns about toxicity (liver fibrosis, hypersensitivity pneumonitis, and teratogenicity), parenteral administration requirements, and the lack of evidence for long term benefit. One long-term benefit study involved placebo controlled withdrawal with 76 patients who had reached remission after 25 mg of methotrexate

intramuscularly weekly for 4-6 months. Forty of those patients then received 15 mg intramuscularly weekly, while the remainder took placebo injections for 40 weeks. It was found more remained in remission on methotrexate (65%) compared to those receiving no treatment (39%). It should be noted those in this study have already shown responsiveness to methotrexate and were able to tolerate the drug at a higher dose.

Effects of Corticosteroids such as Prednisone – Jose

Prednisone is an oral, synthetic corticosteroid used for suppressing inflammatory mechanisms and the immune system. These synthetic corticosteroids mimic the action of cortisol which is naturally produced in the human body by the adrenal glands. As mentioned above, Prednisone is used to treat and manage inflammation or diseases in which the immune system plays a role. It can treat diseases and conditions such as Arthritis, Ulcerative colitis, Crohn's disease, Systemic lupus Allergic reactions, Asthma, and severe Psoriasis. In addition it can also treat Leukemia, Lymphomas, Idiopathic thrombocytopenic purpura, Autoimmune hemolytic anemia, and Bronchitis.

Prednisone is biologically inert and converted to prednisolone in the liver. Prednisone is a glucocorticoid receptor agonist and it is inactive in the body. In order to become active, it first must be converted into Prednisolone by enzymes in the liver. Thus, Prednisone may not work effectively in individuals with liver disease. Prednisolone crosses cell membranes and binds with high affinity to specific cytoplasmic receptors. The result includes inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of corticosteroids are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes. In addition, prednisone has side effects that can weaken the immune system increasing the risk for infection, it is recommended to avoid people who are sick or have been sick in order to prevent infection.

Effect of Antibiotics such as Metronidazole - Huson

One of the main symptoms of Crohn's disease is intestinal inflammation caused by bacteria. Antibiotic treatment is used as one of the main solutions for this inflammation, because it allows for reduction of symptoms and helps to prevent reoccurrence of the symptoms. Antibiotics are used because they are able to reduce the number of intestinal lumen bacteria and alter the bacterial composition to favor only beneficial bacteria. The less bacteria present in the intestines, the less likely they are to penetrate the epithelial cells of follicles in Peyer's patches and induce inflammation. Antibiotics also help to suppress the immune system thereby reducing the immunologic response of inflammation. If the antibiotic medication effectively reduces symptoms, it can be used long term in Crohn's disease patients. One of the most common antibiotics prescribed to CD patients is Metronidazole. It fights a wide range of different bacteria, and as it is used as a primary therapy it is also one of the most extensively studied in medical research for this disease. It is especially an effective treatment after colon resection surgery for the first three months. When compared to the placebo effect, Metronidazole showed to significantly improve symptom recovery. However, Metronidazole also may cause many side effects including gastrointestinal problems and may cause permanent peripheral neuropathy. It has also been

recently hypothesized that the use of antibiotics prior to diagnosis of the disease, particularly at an early life stage, may have actually led to the development of Crohn's disease.

Treatments such as Bowel Resections and Enemas – Christie

Most people who suffer from Crohn's disease will undergo resection surgery to improve their quality of life. Those with Crohn's disease have areas called strictures, which are actively affected by the disease and can be found between sections of healthy bowel. Bowel strictures cause the bowel to become too narrow in that section and result in painful blockages that prevent easy passage of food to the digestive tract. Resection surgery involves removing an area of either the small or large intestine affected by the disease. However, after ileal or ileocolonic resection, there is a 20-30% symptomatic recurrence rate within the first year and a 10% increase each year thereafter. The benefits of resection surgery tend to be temporary depending on various factors. The extensive lesions in the bowel, which can be seen months after surgery, predict rapid evolution to recurrent symptoms and complications. Risk factors for early recurrence are perforating behavior, ileal or ileocolonic resection with ileocolonic anastomosis, and smoking. Preventative measures against the formation of new lesions can be found through drug therapy. Two strategies which tend to interrupt the natural history of Crohn's disease postoperatively are treatments with nitroimidazole antibiotics and immunosuppression therapy.

Another form of relief can be found through enemas, which deliver treatment directly to the inflamed area. Corticosteroid enemas are usually foam-based, and a limited amount of the drug enters the bloodstream. The foam targets various areas such as the colon, descending colon, or rectum. Sodium phosphate enemas are the most common over-the-counter type used to self-treat the symptoms associated with CD. However, there have been no comprehensive studies suggesting this type of enema as an effective treatment for constipation. Overall, the risks which come from use of enemas vary from minimal to high for adverse side effects.

Induction with Infliximab and a Plant-Based Diet as First-Line (IPF) Therapy: A Single-Group Trial

In this single-group trial, the research group claims that biologics are not effective with around 30% Crohn's disease (CD) patients, and they found plant-based diet have never been used to treat in an induction phase of CD. Inflammatory bowel disease (IBD) is a polygenic disease. It could happen under varying circumstances and be triggered by different factors, including the environment. Westernization of lifestyle was recognized to cause IBD, but it's not a recommended solution to change the lifestyle for CD, except quitting smoking. However, consuming meat and sweets was found as a high risk to trigger IBD, and consuming vegetables and fruits proved an effective way to prevent IBD. The research group designed an induction therapy with infliximad combined with a semi-vegetarian diet (SVD), and administered metronidazole 750 mg/d afterward. The whole therapy was performed for a six weeks long period.

The primary endpoint was clinical remission at week 6 after the first infliximab infusion. Clinical remission was defined as the absence of active symptoms. The secondary end points were normalization of C-reactive protein (CRP) concentration at week 6 and mucosal healing. Crohn Disease Activity Index (CDAI) also was evaluated. Patients were morphologically studied with colonoscopy and/or contrast barium enema before discharge. In this study, mucosal healing was defined as the absence of active findings of CD such as ulcer, aphthoid lesions, edema, redness, and bleeding. Symptoms and CDAI were evaluated before and infliximab therapy up to week 6.

In conclusion, IPF therapy can induce remission for most patients with CD regardless of age or new diagnosis or relapse status. It could offers several advantages over current induction therapy. No serious adverse events occurred with IPF therapy. The rapid efficacy of infliximad enabled patients to eat dinner on the same day of infliximad treatment.

Literature Cited

1. Klein, A., Eliakim, R., 2010 Non Steroidal Anti-Inflammatory Drugs and Inflammatory Bowel Disease. *Pharmaceuticals (Basel).* 3:1084-1092.
2. Harbord, M.W.N., Marks, D.J.B., Forbes, A., Bloom, S.L., Day, R.M, Segal,A.W., 2009 Impaired neutrophil chemotaxis in Crohn's disease relates to reduced production of chemokines and can be augmented by granulocyte-colony stimulating factor. *Ailment Pharmacol Ther.* 24:651-660.
3. Wera, O., Lancelloti, P., Oury, C., 2016 The Dual Role of Neutrophils in Inflammatory Bowel Diseases. *J Clin Med.* 5:118.
4. Chiba, M., Tsuji, T., Nakane, K., Tsuda, S., Ishii, H., Ohno, H., Watanabe, K., Ito, M., Komatsu, M., Sugawara, T., 2017 Induction with Infliximab and a Plant-Based Diet as First-Line (IPF) Therapy for Crohn Disease: A Single-Group Trial. *Perm J.* 21-17-009.
5. Nitzan, O., Elias, M., Peretz, A., Saliba, W., 2016 Role of antibiotics for treatment of inflammatory bowel disease. *World J Gastroenterol.* 22:1078-1087.
6. Rugeerts, P., 2002 Crohn's disease recurrence can be prevented after ileal resection. *Gut.* 51:152-153.
7. Rouiller-Braunschweig, C., Fournier, N., Pittet, V., Dudler, J., Michetti, P., 2017 Efficacy, Safety and Mucosal Healing of Methotrexate in a Large Longitudinal Cohort of Inflammatory Bowel Disease Patients. *Digestion.* 96:220-227.
8. Gullberg, E., Soderholm, J.D., 2006 Peyer's Patches and M Cells as Potential Sites of the Inflammatory Onset in Crohn's Disease. *Ann. N.Y. Acad. Sci.* 1072:218-232.
9. Carter, A., 2016 Anti-Inflammatory Drugs for Crohn's. *Healthline.*
10. Hugot, J.P., Alberti, C., Berrebi, D., Bingen, E., Cezard, J.P., 2003 Crohn's disease: the cold chain hypothesis. *The Lancet.* 362: 2012-2105
11. National Center for Biotechnology Information. PubChem Compound Database; CID=5865; https://pubchem.ncbi.nlm.nih.gov/compound/5865
12. Cheifetz, A.S., Rutgeerts, P., Robson, K.M., 2015 Sulfasalazine and 5-aminosalicylates in the treatment of inflammatory bowel disease. *UpToDate.*
13. Kappelman, M.D., Moore, K.R., Allen, J.K., Cook, S.F., 2013 Recent trends in the prevalence of Crohn's disease and ulcerative colitis in a commercially insured U.S. population. *Digestive Diseases and Sciences.* 58:519-525.