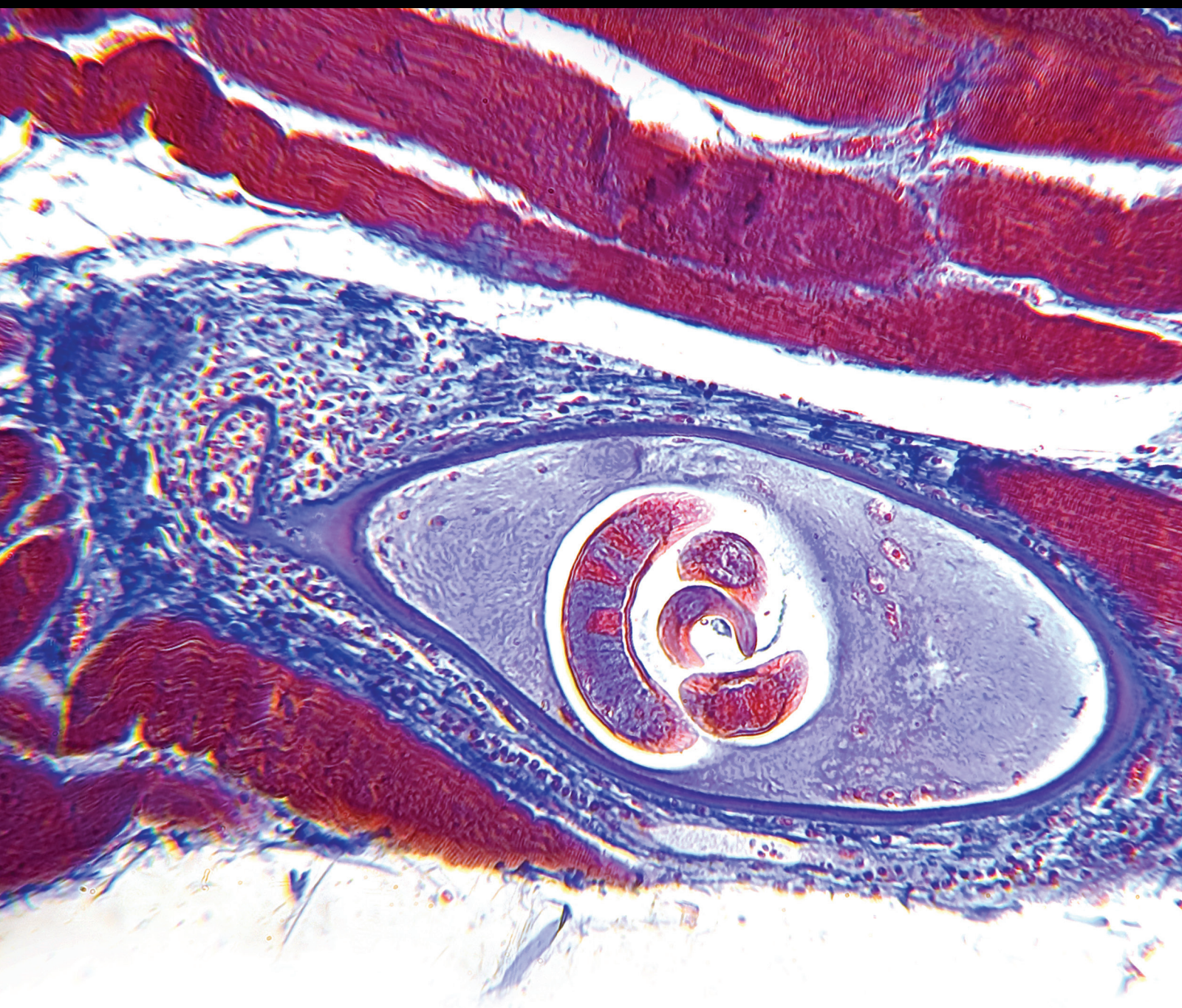
Gastroenterology Research and Practice

Immunological Aspects of Gastrointestinal Diseases

Guest Editors: Shahram Golbabapour, Luísa M. Da Silva, and Antonios Athanasiou



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Editorial Board

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Editorial

Immunological Aspects of Gastrointestinal Diseases

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The gastrointestinal tract is an important organ that directly and indirectly interacts with various organs in the body to produce energy and basic nutrients from food intake. Diseases of gastrointestinal tract not only have various effects on its several functions but endanger patients through its effects on the other organs. Therefore, the maintenance of homeostatic state of this organ is critically important for remaining healthy. In addition to the main physical and chemical functions of the gastrointestinal tract, this organ is a habitat for various microorganisms named microflora which has beneficial functions through a mutually beneficial association with the body. Autoimmune diseases of gastrointestinal system appear with high incidence among adult and children. Primary sclerosing cholangitis is a cholestatic liver disease that causes chronic inflammation of biliary duct. The pathogenesis of this disease is previously well-explained by Pollheimer et al. [1] and Eaton et al. [2]. According to recent research about the role of genetic and immune factors involved in the pathogenesis, primary sclerosing cholangitis appeared to be a common disease among children. Similarly, autoimmune pancreatitis which is a chronic pancreatitis with a high prevalence is one of the important gastrointestinal diseases whose diagnosis is mainly based on immunological parameters [3]. Genetic factors are the main causes of this disease as previously described by Whitcomb [4]. Another chronic inflammation disease in gastrointestinal tract is bowel disease in which the level of interleukin 17 significantly increases [5]. Moreover, host-microbe interaction is crucially important in the incidence of the disease [6] and may attribute in HIV replication as comprehensively reviewed by Brenchley and Douek [7]. The main focus of this special issue is on recent improvements in the immunological aspects of gastrointestinal diseases. Furthermore, gastrointestinal functions and pathophysiology features of gastrointestinal diseases are also reviewed in this special issue.

In the paper entitled "The Role of Genetic and Immune Factors for the Pathogenesis of Primary Sclerosing Cholangitis in Childhood," P. M. Ferri et al. reviewed the effects of immune and genetic factors in the pathogenesis of primary sclerosing cholangitis (PSC) in paediatric patients. The authors discussed that human leukocyte antigen (HLA) class I and class II are the main risk factors for PSC in major histocompatibility complex. Moreover, changes in immune response to pathogens, activation of T lymphocytes, and the release of inflammatory mediators and adhesion molecules contribute to the pathogenesis of PSC as well.

In a review paper entitled "From Pathogenesis, Clinical Manifestation, and Diagnosis to Treatment: An Overview on Autoimmune Pancreatitis," O. Cai and S. Tan discussed the types of autoimmune pancreatitis (AIP) and its classification. Pathogenesis, clinical manifestation, and differential diagnosis of the disease are also discussed in this review paper. Besides, the treatment of AIP disease is elaborated in brief.

In this special issue, another review article entitled "The Immunological Basis of Inflammatory Bowel Disease" is published. In this work, F. A. R. Silva et al. discussed microbiota and genetic factors involved in bowel disease. Moreover, the roles of immune system including innate and

adaptive immunities are summarized in brief. The main cytokines of the adaptive immune response, the effector cells, and their principle actions are explained separately. The importance of T regulatory cells and helper 17 cells in bowel disease is highlighted.

In the paper entitled "The Value of Caspase-3 after the Application of *Annona muricata* Leaf Extract in COLO-205 Colorectal Cancer Cell Line," M. Abdullah et al. evaluated the apoptosis-inducing effect of soursop leaf extract (*Annona muricata*) in COLO-205, a colorectal cancer cell line, through the activities of caspase-3, which is a marker of cell apoptosis. In this study, *Annona muricata* leaf extract showed a better level of caspase-3 as compared with leucovorin (folinic acid) and placebo. The authors concluded that this result may suggest that leaf extract of *Annona muricata* has anticancer properties through enhancing the activity of caspase-3.

In the paper entitled "Investigation of Small Bowel Abnormalities in HIV-Infected Patients Using Capsule Endoscopy," E. Sakai et al. assessed the amendments in intestinal mucosal among HIV patients using capsule endoscopy. The authors concluded that HIV infection itself is able to induce injuries in small bowel mucosal. However, such injuries seem unlikely to cause major clinical symptoms. In addition, HIV infection is closely associated with villous atrophy, which is not completely restored even after the measure of highly active antiretroviral therapy.

Shahram Golbabapour
Luísa M. da Silva
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Research Article

The Value of Caspase-3 after the Application of *Annona muricata* Leaf Extract in COLO-205 Colorectal Cancer Cell Line

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Annona muricata, commonly known as soursop, contains annonacin, acetogenin, and polyphenol which are known to have chemopreventive effects on cancer. In this study, we tend to evaluate the apoptosis-inducing effect of soursop (*Annona muricata*) leaf extract on the colorectal cancer cell line COLO-205 through the activities of caspase-3 which is a marker of cell apoptosis. Cell cultures were incubated with soursop leaf with a concentration of 10 μg/ml and then compared with those of the incubated positive control leucovorin 10 μg/ml and placebo as a negative control. The apoptotic activity of caspase-3 was measured with ELISA. After the administration of each treatment in the colorectal cancer cell line COLO-205, the expression of caspase-3 activity was 1422 ng/ml after incubation with the extract of *Annona muricata* leaves, 1373 ng/ml after the administration of leucovorin, and 1297 ng/ml in the one with placebo. *Annona muricata* leaf extract elevated caspase-3 by 1.09 times compared to that of the pure cell line. *Annona muricata* leaf extract had a higher value of caspase-3 activity than leucovorin and placebo in the COLO-205 colorectal cancer cell line. These results may suggest that *Annona muricata* leaf extract had anticancer properties by enhancing caspase-3 activity which is a proapoptotic marker.

1. Introduction

Cancer is the second leading cause of death in developing countries. These cancer cases are found in developing countries partly due to the diverse populations of age, lifestyle, and environmental factors such as smoking, low-physical activity, Western food, and exposure to variety of jobs [1, 2]. The prevalence of colorectal cancer is increasing in Asia [3]. Colorectal cancer lined up in the third rank of cancers among men and women. Based on the International Cancer Research data, the incidence of colorectal cancer in Asia is similar to that seen in Europe. In the last decade in East Asia such as China, Japan, South Korea, and Singapore, cases of colorectal cancer have increased to two to four times [4]. In Indonesia, colorectal cancer was recently considered one of the most common cancers. From the 13 types of cancers in the registry data, colorectal cancer is one of the five most common cancers found in men and women [5].

There has been approaches using supportive therapy as adjuvant for chemical therapy such as chemotherapy. This supportive therapy can be taken from natural products [6, 7]. Natural product derivatives have flavonoid, terpenoid, and steroid which include cytotoxic and chemopreventive effects [8, 9]. These effects can inhibit tumor initiation, promotion, and progression. Therefore, the scientific validation of traditional medicine should be done for its possible use

in the prevention and treatment of cancer. To fight cancer cells, there is an apoptotic pathway need to be taken by the natural product [6]. Caspase is one of the apoptotic pathways in order to perform cell death. There is overwhelming experimental evidence supporting the idea that inducing caspase response can help cell cancer to become apoptotic [10].

Classification of human caspase is based on their function, size of their prodomain, and cleavage activity. The function of inflammatory caspase is represented by group I caspase, and they are caspases 1, 4, and 5 that are involved in cytokine maturations. Regulations of apoptosis is controlled by group II caspase. There are two classes of group II caspase, and they are the initiator caspases which are performed by caspases 2, 8, 9, and 10 and the effector caspases which are performed by caspases 3, 6, and 7. Effector caspases are produced in cells as dimer and proteolytic processing by an initiator enzyme to trigger its activity. After being active, the effector can target cells leading to program the apoptosis [10].

Caspase-3 is one of the effector caspases that can be measured for the number of apoptosis in cancer cells. Caspase-3 can be initiated by either extrinsic factor or intrinsic factor. Extrinsic factor will initiate FasL and lead to the cleavage of FasR. This leads to the separation of the large and small subunits of catalytic domain. Afterwards, it will activate caspase 8 and induce the activation of caspase-3 which leads to the apoptosis of cells [11].

Nowadays, there are increasing research on natural products to find anticancer activity. One of the anticancer activities can be found from herbal. Herbal with acetogenin activity can be found in the leaves of *Annona muricata* [12, 13]. They contain acetogenins which are known to have cytotoxic effect in tumor cells. Polyphenol activity from *Annona muricata* leaves has chemopreventive effect by lowering the incidence of some types of cancer, especially in colonic epithelial cells [9, 14, 15].

2. Materials and Methods

2.1. Plant Material and Extraction. The material used in this experiment is the soursop leaf extract. The leaves were extracted with a litre of 96% ethanol at room temperature by the Indonesian Institute of Sciences. This results in 1/20 ethanol-soluble material (we called it EIFAM extract). The ethanol-soluble extract was dried until all alcohol contents evaporated (we called it ESFAM extract). For positive control, we used certified standardized material products produced by *Javaplant* which are soluble materials (we called them ORAC extract). We also used 5-fluorouracil and leucovorin for positive control.

2.2. Cell Culture. Cell line used in this study was COLO-205 cell line. COLO-205 was derived from colorectal adenocarcinoma tissues of Dukes of type D with poor differentiation in men aged 70 years from ATCC [16]. COLO cells were grown using DMEM media with 10% FBS, 100 μ/ml antibiotic penicillin streptomycin, and 2 mM L-glutamin. Cells were grown in 75 cm^3 and placed in an incubator with 5% CO_2 concentration and 95% humidity. Before the treated cells were maintained for 3 days until cells reach 90%, cells were then trypsinized using 0.25% trypsin EDTA. Cells were then centrifugated at 1000 rpm for 5 minutes to separate pellets from supernatant. Supernatant was discharged and replaced with new complete media to neutralize the effect of trypsin EDTA.

Table 1: Result of cell viability assay using MTT assay in percentage. This showed that ESFAM extract has the best result compared to others.

	Cell viability (%)
ESFAM	*61.30*
EIFAM	114.21
FU	83.15
Leucovorin	100.71

2.3. Cytotoxicity Assay. The cytotoxicity test was performed using colorimetric assay, MTT assay 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide. The cells were seeded in a 96-well plate with a density of 1×10^5/well using DMEM complete media which contain 10% FBS and 100 μg/ml penicillin streptomycin. After it reaches the confluency, the media were then replaced with another media which contain ORAC extract, EIFAM extract, ESFAM extract, 5-flourouracil, and leucovorin with concentration of 400 ppm, 200 ppm, 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, 3125 ppm, and 15,625 ppm and were then incubated for 24 and 48 h. At the end of each time point, 10 μl of 5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution was added (final concentration 0.5 mg/ml and stock solution 5 mg/ml MTT in PBS) for 4 h. The MTT solution and medium were removed and 100 μl DMSO was added to each well. Absorbance was measured at 570 nm using the ELISA microplate reader.

2.4. Measurement of Caspase-3 Activity. We calculated caspase-3 using ELISA method obtained by R&D System. The plates were conjugated with recombinant caspase-3 and provided with monoclonal antibody raised against caspase-3. All samples, standards, and reagents are performed at room temperature then added to each well for 100 μl each and were incubated at room temperature for 2 hours. After the incubation, each well was aspirated and washed 5 times. The wells were then added with conjugate, 100 μl for each well, and then were incubated at room temperature for 1 hour and proceeded with washing the plates 5 times. Then, the plates were added with substrate solution of 100 μl and were incubated at room temperature for 30 minutes. Stop solution was added to each well and read the plates at 450 nm with correction wavelength of 540 nm. Caspase-3 was available in ng/ml which means the higher value was the better value of caspase-3.

3. Results

3.1. Cell Viability and Cytotoxicity. MTT assay was performed to evaluate the effect of EIFAM, ESFAM, ORAC, 5FU,

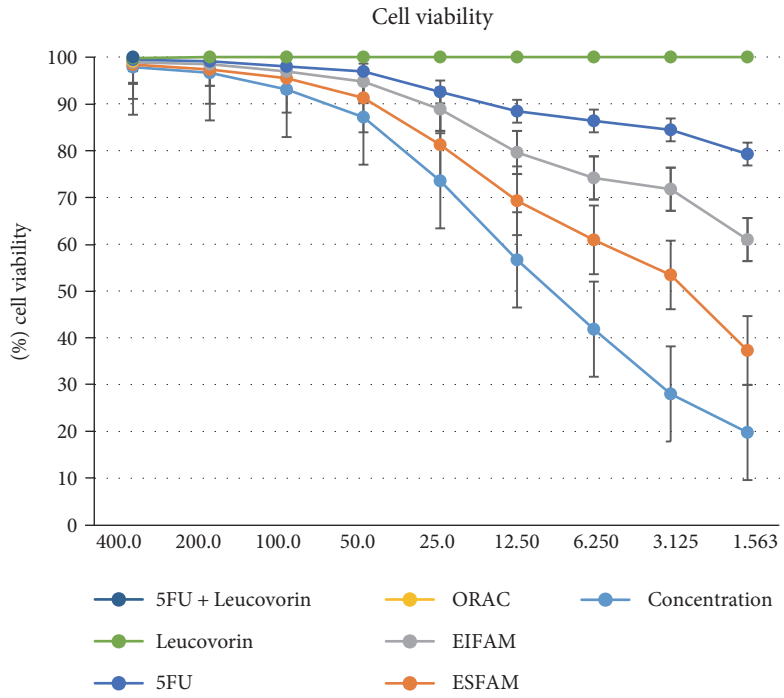

Figure 1: COLO-205 cell lines were treated with ESFAM, EIFAM, leucovorin, and 5FU for 48 h. Cell viability was determined using MTT assay. The results represent the mean ± SD of three independent experiments.

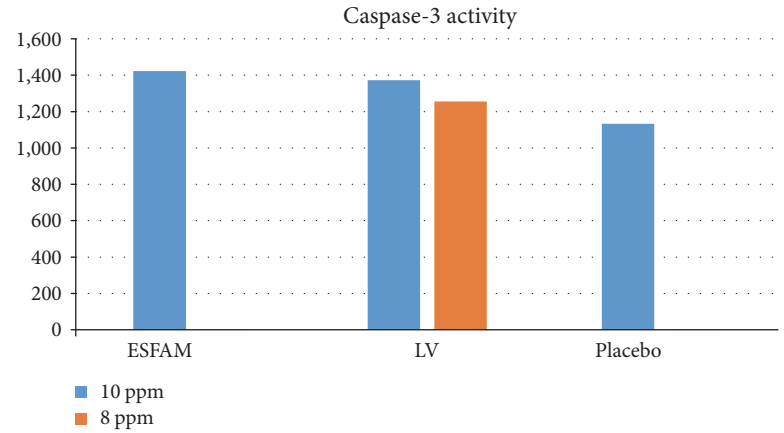

Figure 2: Caspase-3 activity of ESFAM, leucovorin, and placebo with 10 ppm and 8 ppm dosages. ESFAM extract gives a higher result at 10 ppm dosage compared to that at 8 ppm dosage.

leucovorin, and a combination of 5FU and leucovorin (FULV) on the survival of COLO-205 cells (Figure 1). A number of living cells were found in percentage. All extracts were performed three times. From the result, we found that ESFAM has good results compared to the other extracts (Table 1).

From the result, we found that after 48 hours of incubation, cell viability of *Annona muricata* leaf extract was 21.01% whereas leucovorin cell viability was 201.85%. The cell viability was reduced to 61.3% after incubated with *Annona muricata* leaf extract for 48 hours. However, lower cell viability was observed in *Annona muricata* leaf extract compared to that in leucovorin with a significant statistical value ($p < 0.05$). In 48 hours of incubation, the IC-50 of *Annona muricata* leaf extract and leucovorin was 189.6 μg/ml and 87.41 μg/ml, respectively (data not shown).

3.1.1. Caspase-3. For detection of caspase-3 activity, we compared two dosages of each extract and we used 10 μg/ml and 8 μg/ml of dosage for ESFAM, leucovorin, and placebo. From the ELISA result, we found that the ESFAM extract with a concentration of 10 μg/ml gives higher result of caspase, which gives a concentration value of 1422 ng/ml than that of leucovorin and placebo where the values of caspase-3 were 1373 ng/ml and 1297 ng/ml, respectively (Figure 2). *Annona muricata* leaf extract elevated caspase-3 by 1.09 times.

4. Discussion

Increasing incidence of colorectal cancer worldwide needs to be balanced with the development of treatments both chemical and phytochemical [3, 6]. Soursop (*Annona muricata*) leaves contain acetogenins, alkaloids, flavonoids, terpenoids, coumarins, and so on. These components could be found either in a form of water or in ethanol extracts, but in different levels of concentration. Flavonoid level is high in the form of ethanol extracts and low in water. There are a number acetogenins contained in Annonaceae which has anticancer effects [17, 18]. The leaves of *Annona muricata* contain the highest number of flavonols compared to the roots or twigs [19]. The natural component of *flavonolol quercetin* is found mainly in plants and has an anticancer effect [20].

Caspase-3 is the biomarker which is used as a parameter in this study. Caspase-3 is a protease that is most often associated with cell death which catalyzes many cellular proteins and mediates the α-fodrin, gelsolin, and ICAD/DFF-45 which alter the morphology of nuclear and the process of apoptosis [21].

The cytotoxic value of *Annona muricata* leaf extract obtained in this study showed good results and significance based on time which can inhibit cell proliferation up to 45% in 48 hours after the treatment is being given. Through this research, the amount of IC-50 of *Annona muricata* leaf extract in colorectal cancer cell culture COLO-205 was observed. IC-50 is the amount of concentration of *Annona muricata* leaf extract required in inhibiting 50% cell proliferation in vitro. The smaller the number required, the more potent the drug was [22].

The research conducted by Zorofchian Moghadamtousi et al. demonstrated that *Annomuricin E* from *Annona muricata* leaves inhibits the growth of HT-29 cells with IC-50 in the number of $1.62 \pm 0.24\,\mu$g/ml after 48 hours [23]. In another study by Pieme et al., it was found that the IC-50 of *Annona muricata* inhibits the growth of HL-60 cells (leukemia) in the range of 6–12 μg/ml after 48 hours [19]. This shows that a different number of dosage is required in a different type of cancer. Higher concentration of IC-50 was needed in this study compared to that in another study. Different types and different fruits from each country may be different in the concentration of active components that have proapoptotic agent. However, in this study, the value of the active components for proapoptotic function was not measured. In the study conducted by Fidaningsih and Handayani, it is found that the best effect of the water extract of soursop leaves to inhibit proliferation is in the dosage of 75 mg/ml with an 88.45% inhibition rate [24]. On the other hand, in another study by Astirin et al., it is found that the dosage needed for alcohol-soluble soursop leaf extract to achieve the same effect is 2 mg/ml with 35.80% apoptotic figure [25]. Another study which assessed the effect of the leaves of *Annona muricata* against cancer cells described that the leaf extract could induce apoptosis in colon and lung cancer through the mitochondria and has the effect of activation of caspase-3 in leukemia. It is also stated that the anti-inflammatory effect is achieved by lowering the migration of leukocytes and the volume of exudate [26]. In a study conducted by Niu et al., it is found that there was a 1.1 times increase in caspase-3 in COLO-205 which was mediated through mitochondria [20]. However, Mondal et al. found no effects of elevation of caspase-3 with the intervention of *Annona* [27].The same result was obtained by Yuan et al. who stated that there was a decrease of activity of procaspase-3 after 8 hours of administration of annonacin, a type of acetogenin [28].

In this study, *Annona muricata* leaf extract had a 1.09 times higher caspase-3 activity compared to the placebo and 1.03 times higher compared to the leucovorin. Leucovorin acts as precursor for 5,10-methylenetetrahydrofolate, which is needed to form the ternary complex with FdUMP (contained in 5-flourouracil) and thymidylate synthase (TS, which is an important target of chemotherapy), is essential for long-term maintenance of TS inhibition. Leucovorin effect can also increase the availability of CH2-THF which will be polyglutamylated and will trigger the inhibition of TS. With the absence of CH2-THF or one of the polyglutamates, FdUMP forms an unstable binary complex, which results in poor inhibition [29, 30].

On the other side, most acetogenin annonacea especially *Annona muricata* was defined by between one and three THF rings with one or two hydroxyl groups on the long-chain hydrocarbon [31]. *Annona muricata* has an adjacent bis-tetrahydrofuran ring system which has the most powerful potential cytotoxicity [32, 33]. This result could suggest the role of *Annona muricata* as combination therapy such as leucovorin.

5. Conclusion

Annona muricata leaf extract had a better value of caspase-3 compared to leucovorin and placebo. These results may suggest that *Annona muricata* leaf extract had anticancer properties by enhancing the caspase-3 activity which is a proapoptotic marker. However, further research to determine which of the active fraction of *Annona muricata* leaves has the effect of increasing the activity of caspase-3 is of great demand. It will enrich the scientific evidence of *Annona muricata* leaf activity as a proapoptotic agent for cancer.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Investigation of Small Bowel Abnormalities in HIV-Infected Patients Using Capsule Endoscopy

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HIV infection is reportedly associated with an increased permeability of the intestinal epithelium and can cause HIV enteropathy, which occurs independently of opportunistic infections. However, the characteristics of small bowel abnormalities attributable to HIV infection are rarely investigated. In the present study, we assessed the intestinal mucosal changes found in HIV-infected patients and compared them with the mucosa of healthy control subjects using capsule endoscopy (CE). Three of the 27 HIV-infected patients harbored gastrointestinal opportunistic infections and were thus excluded from subsequent analyses. The endoscopic findings of CE in HIV-infected patients were significantly higher than those in control subjects (55% versus 10%, $P = 0.002$); however, most lesions, such as red spots or tiny erosions, were unlikely to cause abdominal symptoms. After validating the efficacy of CE for the diagnosis of villous atrophy, we found that the prevalence of villous atrophy was 54% (13/24) among HIV-infected patients. Interestingly, villous atrophy persisted in patients receiving long-term antiretroviral therapy, though most of them exhibited reconstituted peripheral blood CD4+ T cells. Although we could not draw any conclusions regarding the development of small bowel abnormalities in HIV-infected patients, our results may provide some insight regarding the pathogenesis of HIV enteropathy.

1. Introduction

The management of opportunistic infections of the gastrointestinal tract is crucial for improving the morbidity and mortality rates of AIDS patients. Since the introduction of highly active antiretroviral therapy (HAART), the frequency of opportunistic infections has been substantially reduced [1]. Meanwhile, HIV itself has been regarded as a mediator of small bowel enteropathy. As the lymphoid tissue of the gut plays an important role in the defense against external pathogens, the gastrointestinal mucosa can become the main target of HIV infection [2, 3]. In addition, the function of the intestinal epithelial barrier is closely associated with progressive HIV replication [4]. Previous reports have suggested that intestinal mucosal barrier defects occur independently of opportunistic infections [5–7], reflecting the impact of HIV infection itself. Therefore, early gastrointestinal mucosal events should be carefully examined to better understand the pathogenesis of HIV infection. Crypt hyperproliferation and villous shortening, resulting in partial villous atrophy, reportedly occur as specific morphological features of HIV enteropathy and can be observed at all stages of HIV infection [8–10]. However, most investigations have only examined the duodenum, because of the difficulty in accessing the small bowel. Therefore, small intestinal abnormalities attributed to HIV infection remain poorly characterized.

Capsule endoscopy (CE) was first introduced in 2000 [11] and has since become established as a useful modality for diagnosing small bowel abnormalities [12–15]. CE enables the entire small bowel to be visualized at a magnification in a minimally invasive manner. Although CE is predominantly used for patients with obscure gastrointestinal bleeding

(OGIB), its usefulness has also been demonstrated in patients with celiac disease (CD), which is an immune-mediated disorder occurring in people genetically susceptible to gluten [16]. Because villous atrophy is frequently observed in both CD and HIV-infected patients, we speculated that CE examination might be useful for revealing the characteristics of HIV enteropathy.

Since the present study was conducted to reveal mucosal changes attributed to HIV infection itself, we first performed an entire gastrointestinal endoscopic examination and excluded patients with specific opportunistic gastrointestinal infectious diseases. Subsequently, we confirmed the validity of the application of CE for the diagnosis of villous atrophy in HIV-infected patients. The characteristics of small bowel abnormalities were compared between HIV-infected patients and healthy control subjects. In addition, we investigated the correlation between clinical parameters related to HIV infection and small bowel abnormalities. Our results will provide insight into the details of HIV enteropathy.

2. Material and Methods

2.1. Patients. Between May 2007 and October 2014, 27 consecutive HIV-infected patients who underwent CE at Yokohama City University Hospital were enrolled in this study. All of the patients had undergone upper and lower endoscopic examinations prior to the CE. As this study aimed to reveal mucosal changes attributable to HIV infection itself, patients with specific opportunistic infectious diseases (e.g., infection with cytomegalovirus (CMV), mycobacteriosis, cryptosporidium, or tuberculosis) were excluded. In addition, a fecal culture was performed to exclude bacterial enteritis (e.g., *Salmonella* spp., *Escherichia coli*, and *Campylobacter* spp.). Moreover, patients using aspirin and/or nonsteroidal anti-inflammatory drugs were excluded, because such drugs can induce small bowel injury [13, 15]. A total of 21 healthy adult subjects were also included as a control group for the comparison of small bowel abnormalities. We registered the patient data, including the age, sex, smoking history, alcohol history, hemoglobin concentration, and albumin and CRP values. Clinical symptoms (abdominal pain, diarrhea, and gastrointestinal bleeding) and the details of HIV infection (history of antiretroviral therapy, follow-up duration, viral load, and peripheral blood CD4 count) were also evaluated at the time of the initial CE. The antiretroviral therapy consisted of a standard combination of two nucleoside reverse transcriptase inhibitors together with either a nonnucleoside reverse transcriptase inhibitor or a protease inhibitor (HAART). The study protocol was approved by the Ethics Committee of Yokohama City University Hospital. Written informed consent was obtained from all of the subjects prior to their participation in the study.

2.2. Capsule Endoscopy. The protocol for the CE procedure has been previously reported [13, 14]. In summary, the patients were instructed to swallow the CE capsule (PillCam SB/SB2; Given Imaging, Yoqneam, Israel) in a solution of dimethicone after fasting overnight, with no other bowel preparation. They were allowed to drink clear liquids 2 hours after they had swallowed the capsule and to eat a light meal 4 hours after. After 8 hours, we confirmed whether the capsule had passed through the ileocecal valve using the Real-Time Viewer (Given Imaging) and the examination was continued to achieve complete entire small bowel visualization. Two CE experts (with experience evaluating more than 150 CE videos) separately read and interpreted the complete CE videos. When discrepancies in interpretation occurred, both experts reviewed the findings simultaneously and reached a consensus. Each of the CE videos was divided into two segments of equal length according to the small-bowel transit time: the first segment was considered to represent the proximal small bowel, and the second to represent the distal small bowel.

2.3. Definition of Small Bowel Villous Atrophy. The duodenal mucosal pathology was evaluated by an experienced pathologist and was used as the gold standard for the diagnosis of villous atrophy. At least 4 biopsies were performed in the lower duodenum. The specimens were cut longitudinally at $4\,\mu$m and stained with hematoxylin and eosin. According to the modified Marsh classification [17], grade ≥ 3 was regarded as being positive for villous atrophy. Villous atrophy was endoscopically diagnosed as positive when the following features were found using CE in the duodenum: reduction or absence of Kerckring's folds, mosaic mucosal pattern, and scalloping [16]. To validate the application of CE to the diagnosis of villous atrophy, the sensitivity, specificity, and positive predictive value were evaluated. In addition, the time until the appearance of the first villi and time with features of atrophy were also recorded, expressed in hours and fraction of hours [18].

2.4. Outcomes after CE Examination. For patients with persisting symptoms in which the CE examination identified severe significant abnormalities, a subsequent balloon-assisted endoscopy (BAE) was performed as a therapeutic intervention or for diagnostic biopsy, where necessary. To reveal the association between villous atrophy and HIV infection, the expression of intestinal CD4+ T cells was examined using duodenal specimens and immunohistochemistry staining (NCL-L-CD4-368; Novocastra, Newcastle, United Kingdom).

2.5. Statistical Analysis. All the data were presented as the mean $\pm$ standard deviation, unless otherwise specified. The statistical significances of the differences in the values of the clinical parameters were evaluated using Fisher's exact test and an unpaired Student's t-test. The P value was 2 sided, and $P < 0.05$ was used to determine statistical significance. All the analyses were performed using the SPSS, ver. 11.0 (SPSS Inc., Chicago IL, USA).

3. Results

3.1. Characteristics of Enrolled Patients. The demographic and clinical characteristics of the enrolled patients are shown in Table 1. Most of the HIV-infected patients were males (96%) and were significantly older than the control subjects (45.0 ± 10.2 years versus 32.2 ± 4.3 years, $P < 0.001$). As for clinical symptoms, diarrhea was commonly present in HIV-

Table 1: Characteristics of enrolled patients.

	HIV-infected patients	Control subjects	P value
Number	27	21	
Sex			
Male	26 (96%)	19 (90%)	0.57
Female	1 (4%)	2 (10%)	
Age, mean ± SD, year	45.0 ± 10.2	32.2 ± 4.3	<0.001
Smoking habit	9 (33%)	6 (29%)	0.76
Alcohol intake	8 (30%)	8 (38%)	0.56
Clinical symptoms			
Abdominal pain	2 (7%)	0 (0%)	0.50
Diarrhea	16 (63%)	0 (0%)	<0.001
Gastrointestinal bleeding	2 (7%)	0 (0%)	0.50
Duration after initial diagnosis, median (range), year	4.0 (0–20)	n.a.	n.a.
HAART	18 (67%)	n.a.	n.a.

HAART: highly active antienteroviral therapy; P values were analyzed using Fisher's exact test or Student's t-test for age.

infected patients (63% versus 0%, $P < 0.001$). Among the 27 HIV-infected patients, 18 (67%) had received HAART and their viral loads were suppressed below the limit of detection. Meanwhile, 6 (22%) patients underwent CE at the time of the initial diagnosis of HIV infection. The median follow-up duration after the initial diagnosis was 4.0 years (range, 0–20 years). An entire small bowel examination was successfully achieved in most of the case, while the CE capsule failed to reach the cecum in only one case with CMV enteritis.

3.2. HIV-Infected Patients with Specific Opportunistic Infections. A subsequent BAE and pathological examination revealed that three of the 27 HIV-infected patients harbored gastrointestinal opportunistic infections. One patient with OGIB who exhibited multiple small bowel ulcers was diagnosed as having CMV-induced small bowel enteritis and began receiving ganciclovir therapy. The other patients with chronic diarrhea were diagnosed as having either jejunal Kaposi's sarcoma or small bowel mycobacteriosis. These patients had been untreated and therefore subsequently received HAART after the opportunistic infections had been controlled. As this study aimed to reveal the mucosal changes attributable to HIV infection itself, these patients were excluded. Finally, a total of 24 HIV-infected patients were eligible for the subsequent analyses.

3.3. Validity of the Diagnosis for the Villous Atrophy Using CE. Representative images of villous atrophy are presented in Figure 1. Of the 15 HIV-infected patients who underwent a histopathological evaluation of the duodenal mucosa, endoscopic markers of villous atrophy were identified in 47% (7/15) of the cases. When the pathological diagnosis was used as the gold standard, the sensitivity, specificity, and positive predictive value for the diagnosis of villous atrophy using CE were 100%, 89%, and 86%, respectively (Table 2).

3.4. Small Bowel Abnormalities Identified in HIV-Infected Patients. The comparison of small bowel abnormalities between HIV-infected patients and control subjects is summarized in Table 3. The diagnostic yield of CE in HIV-infected patients was significantly higher than that among the control subjects (55% versus 10%, $P = 0.002$). The prevalence and number of erosions were also significantly higher among HIV-infected patients (42% versus 5%, $P = 0.01$, and 0.8 ± 0.6 versus 0.1 ± 0.1, $P = 0.02$), while ulceration was rarely identified in both groups (13% versus 0%, $P = 0.24$). There were no significant differences in the distributions of small bowel abnormalities. Other significant abnormalities, such as angioectasia and tumors, were not identified in either group. The prevalence and number of small bowel abnormalities were not different between patients with and those without a peripheral blood CD4 count < 200/μL (71% versus 47%, $P = 0.39$, and 1.5 ± 1.5 versus 1.0 ± 1.4, $P = 0.50$).

Since the efficacy of the endoscopic diagnosis of villous atrophy was demonstrated, we assessed the prevalence of villous atrophy among all the enrolled patients. Although no endoscopic markers of villous atrophy were found in the control subjects, markers were identified in 54% (13/24) of the HIV-infected patients. A reduction or absence of Kerckring's folds was frequently observed (11/13, 85%), while a mosaic mucosal pattern and scalloping were rarely identified (2/13, 15%). Among 13 HIV-infected patients with villous atrophy, the time to the appearance of first villi was 0.3 ± 0.6 hours. The mean time with features of villous atrophy was 0.5 ± 0.4 hours. When the distribution of the villous atrophy was evaluated, the proximal small bowel mucosa was predominantly involved (21% versus 6%).

3.5. Association between HIV Infection and Villous Atrophy. A comparison of the clinical parameters according to the presence of villous atrophy is presented in Table 4. There were no significant differences in the hemoglobin concentration or the serum albumin and/or CRP values. Diarrhea was frequently present in patients with villous atrophy, but the significance was borderline (77% versus 36%, $P = 0.10$). Antiretroviral therapy was equally used in both villous atrophy positive and negative patients (77% and 73%, $P = 0.81$). There was no significant difference in the presence of villous atrophy when compared between patients with and without a peripheral blood CD4 count < 200/μL (38% versus 18%, $P = 0.28$). In addition, the intestinal CD4+ cell expression level was also similar.

4. Discussion

In the present study, we investigated the details of small bowel abnormalities in HIV-infected patients using CE. Based on the results of entire gastrointestinal endoscopic examinations and subsequent pathological evaluations, we identified three (11%) cases of opportunistic infectious disease. These cases occurred in symptomatic naïve HIV-

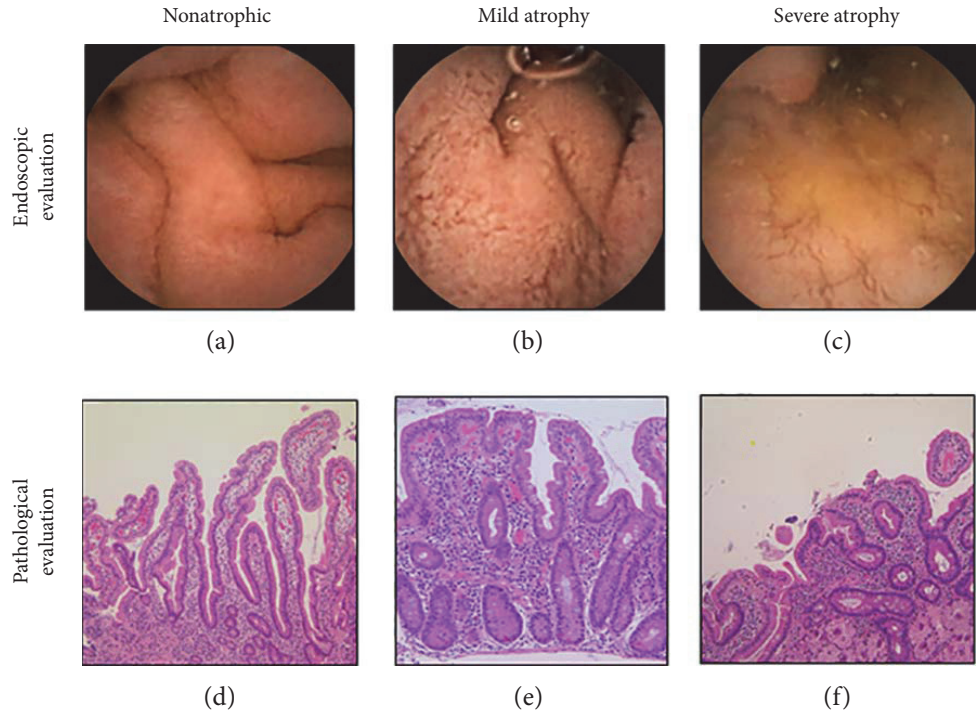

Figure 1: Representative images of villous atrophy. Villous atrophy was endoscopically diagnosed as positive when the reduction or absence of Kerckring's folds, a mosaic mucosal pattern and scalloping, was confirmed in the duodenum (a–c). A biopsy was performed at the lower duodenum, and villous atrophy was evaluated using the modified Marsh classification [17] (d–f). Marsh stage ≥ 3 was diagnosed as villous atrophy positive. (a) Nonatrophic villi. (b) Reduction of villi. (c) Mosaic pattern of mucosa. (d) Marsh 0. (e) Marsh 3a. (f) Marsh 3c.

Table 2: Correlation between endoscopic and pathological findings of small bowel atrophy.

	Capsule endoscopy evaluation	Pathological evaluation
Atrophy positive	7	6
Atrophy negative	8	9

The sensitivity, specificity, and positive predictive value for the diagnosis of villous atrophy using capsule endoscopy were 100%, 89%, and 86%, respectively.

infected patients, thus necessary to doubt harboring opportunistic infections. Importantly, all the infectious foci were limited to the small bowel, which would have made early detection difficult even after a conventional gastrointestinal endoscopic examination. Moreover, Mönkemüller et al. reported that approximately 10% of HIV-infected patients taking HAART can present with gastrointestinal opportunistic infections [19]. Therefore, the application of CE should be carefully considered, if the possible presence of gastrointestinal opportunistic infections is suspected.

Oette et al. investigated gastrointestinal abnormalities in HIV-infected patients and reported that a number of findings with therapeutic implications were identified in the small bowel [20]. Of note, most of their patients were markedly immunosuppressed (peripheral blood CD4 count < 200/μL); therefore, the effects of the HIV infection itself could not be discussed. Unlike their report, we included patients whose viral replication had been intensively

Table 3: Comparison of small bowel abnormalities diagnosed by capsule endoscopy.

	HIV-infected patients	Control subjects	*P* value
Number	24	21	
Diagnostic yield, *N* (%)	13 (55%)	2 (10%)	0.002
Redness, prevalence, *N* (%)	7 (29%)	2 (10%)	0.14
Total, *N* (mean)	10 (0.4)	3 (0.1)	0.17
Proximal, *N* (mean)	5 (0.2)	0	0.06
Distal, *N* (mean)	5 (0.2)	3 (0.1)	0.63
Erosion, prevalence, *N* (%)	10 (42%)	1 (5%)	0.01
Total, *N* (mean)	18 (0.8)	2 (0.1)	0.02
Proximal, *N* (mean)	11 (0.5)	1 (0.1)	0.01
Distal, *N* (mean)	7 (0.3)	1 (0.1)	0.19
Ulceration, prevalence, *N* (%)	3 (13%)	0	0.24
Total number, *N* (%)	4 (0.2)	0	0.10
Proximal, *N* (mean)	2 (0.1)	0	0.36
Distal, *N* (mean)	2 (0.1)	0	0.36
Villous atrophy, *N* (%)	13 (54%)	0	<0.001

Among 27 HIV-infected patients, three patients diagnosed as having Kaposi's sarcoma, small bowel mycobacteriosis, and cytomegarovirus-induced small bowel enteritis, respectively, were excluded from subsequent analyses. Each of the CE videos was divided into two segments of equal length according to the small-bowel transit time; the first segment was considered as representing the proximal small bowel, and the second as representing the distal small bowel. *P* values were analyzed using Fisher's exact test or Student's *t*-test.

Table 4: Association between clinical factors and villous atrophy.

	Villous atrophy (+)	Villous atrophy (−)	*P* value
Number	13	11	
Age, year	43.8 ± 11.1	44.9 ± 8.8	0.80
Duration after initial diagnosis, median (range), year	4.0 (0–9.0)	4.0 (0–20.0)	0.35
Clinical symptoms			
Abdominal pain	1 (8%)	0 (0%)	>0.99
Diarrhea	10 (77%)	4 (36%)	0.10
Serum albumin value, g/dL	4.4 ± 0.5	4.4 ± 0.6	0.89
Hemoglobin value, g/dL	13.8 ± 1.9	14.1 ± 1.5	0.34
CRP, mg/dL	0.2 ± 0.3	0.2 ± 0.3	0.65
HAART therapy	10 (77%)	8 (73%)	0.81
Peripheral blood CD4 count < 200/μL	5 (38%)	2 (18%)	0.28

HAART: highly active antienteroviral therapy; *P* values were analyzed using Fisher's exact test or Student's *t*-test for age and the Mann-Whitney *U* test for follow-up duration.

suppressed using HAART (67%); therefore, only a few findings with therapeutic implications were identified. Active HIV replication leads to a local increase in inflammatory cytokine, such as TNFα, and impairs mucosal barrier function [21]. The degree of inflammation within the gut is reportedly correlated with viral replication [22]. Epple et al. reported that the suppression of HIV replication by HAART improved mucosal barrier defects [23]. Although we confirmed a significantly higher diagnostic yield of CE among HIV-infected patients, most of the small bowel abnormalities identified in whom seem less relevant (e.g., red spots and/or tiny erosions). In addition, we confirmed that the presence of small bowel injuries did not differ between treated and untreated HIV patients. Taken together, we consider that external pathogens are probably required to cause significant small bowel damages, though the mucosal barrier defect itself can be attributed to HIV infection.

Previous reports have indicated a potential role for CE as a diagnostic tool for villous atrophy [16, 24]. In a recent meta-analysis assessing the accuracy of CE as a diagnostic tool in CD, the sensitivity of this modality was 89%, with a specificity of 95% [25]. In addition, CE is reportedly useful for accessing the therapeutic response in CD patients [26]. Our results suggested that CE might also be useful for assessing the presence of villous atrophy in HIV-infected patients. We revealed that the proximal small bowel was likely to present villous atrophy. Although the distribution of villous atrophy was similar to CD patients [16], the levels of villous atrophy seemed comparatively low among the HIV-infected patients, since a scalloping and/or mosaic pattern (which is often observed in CD patients) was rarely identified. Consistent with our report, Troeger et al. also confirmed that severe villous atrophy is rarely seen in HIV-infected patients, despite the resemblance of the histological changes in small bowel structures [27]. This finding probably reflects the fact that most HIV-infected patients continue to have a good nutritional condition, despite having a high frequency of villous atrophy.

Gastrointestinal symptoms reportedly improve soon after the introduction of HAART [28], suggesting that HAART can ameliorate enteropathy. However, we confirmed that 36% of the patients treated with HAART still presented with diarrhea. Although other causes of diarrhea (e.g., drug-induced) should be ruled out, we believe that the presence of diarrhea might be partly associated with villous atrophy. Importantly, villous atrophy was still observed in patients who had received long-term HAART, though most of them exhibited reconstituted peripheral blood CD4+ T cells. Meanwhile, HIV-infected patients rarely exhibit a reconstituted CD4+ T cell population in the small bowel, possibly because viral replication continues at a low level, even after the introduction of HAART [29]. Guadalupe et al. reported that genes involved in inflammation and stress were upregulated in such patients, contrary to the decreased expression of genes involved in digestive and absorptive functions [30]. These mucosal conditions are thought to help the sustained transformation of the intestinal mucosa, resulting in partial villous atrophy. Unfortunately, we could not confirm an association between villous atrophy and intestinal CD4+ T cell expression, probably because of the limited number of available samples. On the other hand, Batman et al. reported that HAART can restore normal crypt structure by inhibiting HIV-driven stem cell hyperproliferation at the crypt bases [31]. To confirm the pathogenesis of HIV enteropathy, additional investigations, such as flow cytometry and/or western blot analyses, are needed. In addition, multifocal small bowel biopsies may help to understand the pathogenesis of small bowel abnormalities, including villous atrophy and mucosal injuries.

The present study had several limitations. First, we lacked adequate control patients with persistent symptoms and instead used nonsymptomatic healthy subjects. In addition, male patients were predominantly enrolled in this study. These factors could potentially affect the validity of our findings. Second, the overall number of patients included in the study was comparatively small. Third, despite the clear correlation between pathological and endoscopic findings obtained from distal duodenum, we could not assess the specimen obtained from middle to distal small bowel. Fourth, we did not perform the follow-up CE examinations, which

could have limited our conclusions. Finally, unspecific CE findings may not be regarded as HIV-associated disease, though we tried to exclude patients with external gastrointestinal infectious diseases and/or drug-induced small bowel injury. Further studies are needed to clarify the relevance of the spectrum of pathological results.

5. Conclusions

Our results indicated that HIV infection itself can induce small bowel mucosal injury; however, such injury is unlikely to cause clinical symptoms. In addition, we also revealed that HIV infection is closely associated with villous atrophy, which is not completely restored even after HAART. Although we could not make any conclusions regarding the pathogenesis of small bowel abnormalities in HIV-infected patients, previous reports highlight the importance of mucosal barrier impairment caused by HIV infection itself. Since the gastrointestinal mucosa is the main target of HIV infection, CE examination may help to better understand the pathogenesis of HIV infection.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review Article

From Pathogenesis, Clinical Manifestation, and Diagnosis to Treatment: An Overview on Autoimmune Pancreatitis

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Autoimmune pancreatitis (AIP) is a special type of chronic pancreatitis which is autoimmune mediated. The international consensus diagnostic criteria (ICDC) 2011 proposed two types of AIP: type I is associated with histological pattern of lymphoplasmacytic sclerosing pancreatitis (LPSP), characterized by serum IgG4 elevation, whereas type 2 is named idiopathic duct-centric pancreatitis (IDCP), with granulocytic epithelial lesion (GEL) and immunoglobulin G4 (IgG4) negative. The pathogenic mechanism is unclear now; based on genetic factors, disease specific or related antigens, innate and adaptive immunity may be involved. The most common clinical manifestations of AIP are obstructive jaundice and upper abdominal pain. The diagnosis can be made by a combination of parenchymal and ductal imaging, serum IgG4 concentrations, pancreatic histology, extrapancreatic disease, and glucocorticoid responsiveness according to ICDC 2011. Because of the clinical and imaging similarities with pancreatic cancer, general work-up should be done carefully to exclude pancreatic malignant tumor before empirical trial of glucocorticoid treatment. Glucocorticoid is the most common drug for AIP to induce remission, while there still exists controversy on steroid maintenance and treatment for relapse. Further studies should be done to identify more specific serum biomarkers for AIP, the pathogenic mechanisms, and the treatment for relapse.

1. Introduction

Autoimmune pancreatitis (AIP) is a special form of chronic pancreatitis that is autoimmune mediated [1]. Autoimmunity is defined as acquired immune reactivity against self-antigens. Autoimmune diseases (AIDs) occur when autoimmune responses lead to tissue damage. AIDs are often classified into two patterns; some are organ specific, for example, diabetes mellitus, in which the pancreas is the target organ, whereas others are systemic, for example, systemic lupus erythematosus (SLE), in which many tissues and organs of the body are damaged. Some common AIDs include diabetes mellitus type 1, Grave's disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and SLE. AIP belongs to and shares some characteristics with AID in pathophysiology, clinical manifestations, and treatment and of course has its uniqueness. The prevalence rate of AIP in Japan was 4.6 per 100,000 individuals in 2011 and the annual incidence rate was 1.4 per 100,000 individuals [2]. In 1961, Sarles et al. [3] first reported a case about nonalcoholic chronic pancreatitis accompanied by hypergammaglobulinemia and predicted its association with an autoimmune process. In 1995, Yoshida et al. [4] first proposed the clinical entity of autoimmune pancreatitis. From then on, more and more scholars have paid attention to this rare type of chronic pancreatitis and substantial progress has been made in the recognition of AIP. The international consensus diagnostic criteria (ICDC) 2011 [5] proposed two forms of AIP: type I is associated with histological pattern of lymphoplasmacytic sclerosing pancreatitis (LPSP), accompanied with the serum immunoglobulin G4 (IgG4) elevation, whereas type 2 is characterized by idiopathic duct-centric pancreatitis (IDCP), with granulocytic epithelial lesion (GEL) and IgG4 negative [5, 6]. The diagnosis of AIP depends on serum IgG4 concentration, pancreatic histology, pancreatic parenchymal and duct imaging, other organ involvement, and steroid reaction and is most often confused with pancreatic cancer, especially the focal AIP exhibiting mass formation [5, 7, 8]. Therefore, some patients

with focal AIP have undergone surgical resection due to the suspicion of malignancy, despite recent improvements in radiological imaging modalities [9–13]. Kobayashi et al. [8] reported 11 (72.2%) AIP patients had undergone surgery due to a preoperative diagnosis of mass formation pancreatitis with possible cancer revealed to be focal AIP. Hence, we sought to prepare an updated review about AIP to get a comprehensive knowledge about it.

2. Classification

The international consensus diagnostic criteria (ICDC) 2011 [5] had classified AIP into two types. Type 1 called lymphoplasmacytic sclerosing pancreatitis (LPSP), or without granulocyte epithelial lesions (GELs), has some characteristic features in histopathology: dense infiltration of plasma cells and lymphocytes; peculiar storiform fibrosis; obliterative phlebitis [15]; elevated IgG4-positive plasma cells (generally >50 cells per high-power field [HPF] [16]). It generally is believed to be the pancreatic manifestation of an IgG4 related systematic disease and is often accompanied with some extrapancreatic lesions, such as sclerosing cholangitis, sclerosing sialadenitis, and retroperitoneal fibrosis [5, 15, 17]. This type of AIP usually presents with obstructive jaundice in elderly male subjects and responds well to steroid therapy [2, 5, 18].

Type 2 called idiopathic duct-centric pancreatitis (IDCP) has the unique characteristic feature of intraluminal and intraepithelial neutrophils in medium-sized and small ducts as well as in acini in histopathology, which is not seen in LPSP [5]. Also, they share some features in histopathology, such as periductal lymphoplasmacytic infiltration and storiform fibrosis. IDCP often has no or few IgG4-positive cells (<10 cells/HPF) and it seems to be a pancreatic-specific disorder, because it is IgG4 negative and is not associated with other organ involvement (OOI) [5]. Patients in IDCP are often a decade younger and do no show gender preference. IDCP lacks a serological marker and for its diagnosis pancreatic histology is a must [5]. The comparisons between the two types of AIP are in Table 1.

Table 1: Comparisons of the two types of AIP.

Characteristics	Type 1	Type 2
Other nomenclatures [5]	LPSP AIP without GEL IgG4 related	IDCP AIP with GEL IgG4 unrelated
Ethnic [5]	Asia > United States, Europe	Europe > United States > Asian
Age [2, 5, 18]	60 years or older	A decade younger
Sex [5]	Usually male	Equal
Symptom [5]	Obstructive jaundice often Abdominal pain rare Pancreas swelling common	Obstructive jaundice often Abdominal pain common Pancreas swelling common
Serology [2, 5]	High serum IgG4, auto-Ab+	Normal serum IgG4, auto-Ab−
Histopathology [5]	Lymphocyte and plasmacyte infiltration and fibrosis Infiltration of IgG4 plasma cells	Granulocyte epithelial lesion often with destruction and obliteration of the pancreatic duct
Extrapancreatic lesion [5, 15, 17]	Sclerosing cholangitis Sclerosing sialadenitis Retroperitoneal fibrosis, etc.	Unrelated with OOI
Ulcerative colitis [2, 5]	Rare	Often
Histology needed for diagnosis [5]	No	Yes
Respond to steroid [2, 5]	Responsive	Responsive
Relapse rate [5]	High	Low

3. Pathogenesis

Recent studies have suggested several possible pathogenic factors in the development of AIP, though its pathogenic mechanism remains unclear. Based on genetic factors, disease specific or related antigens, innate and adaptive immunity may be involved [19].

3.1. Genetic Factors. Kawa et al. [20] first revealed that the susceptibility of AIP in Japanese patients may be associated with class II antigen haplotype of the major histocompatibility complex (HLA-DRB1*0405-DQB1*0401). Later, Umemura et al. found that serum IgG4 concentrations in Japanese patients with AIP were significantly positively correlated with the number of susceptible Fc receptor-like 3 (FCRL3) genes alleles [21] expressed on B cells in 2006 and cytotoxic T lymphocyte antigen 4 (CTLA-4) [22] expressed on $CD4^+$ and $CD8^+$ T cells in 2008. In 2011, Ota et al. evaluated the association of AIP with single nucleotide polymorphisms (SNPs) and provided the evidence of KCNA3 [23] association with AIP. Chang et al. revealed the association of cystic fibrosis transmembrane conductance regulator (CFTR) gene variants [24] with AIP. Although the functions of the CFTR variants and their roles in the pathogenesis of AIP were not elucidated that clear, CFTR variants may play roles as disease modifiers in AIP (seen in Table 2). Undeniably, FCRL3 is found to be associated with various autoimmune diseases, such as rheumatoid arthritis, autoimmune thyroid disease, and SLE in Japanese populations [25, 26].

3.2. Immunogenic Factors. AIP is an autoimmune-mediated disease and abnormal immune response may play an important role in its pathophysiology. More than one autoantibody is seen in AIP patients and some other antigens like lactoferrin (LF), carbonic anhydrase (CA) II [27, 28], pancreatic secretory trypsin inhibitor (PSTI) [29], amylase alpha 2A [30], and type IV collagen [31] may also be involved in the

Table 2: Genetic factors in the pathogenesis of AIP.

Gene related	Cells involved	Sites related	Possible function in AIP	Referencing
HLA-DRB1*0405-DQB1*0401	T cells	HLA-DRB1*0405-DQB1*0401 haplotype	Inducing an autoimmune response; genetic marker for non-HLA gene associated disease susceptibility	Kawa et al. [20]
FCRL3	B cells	FCRL3-110 alleles	Susceptibility with AIP	Umemura et al. [21]
CTLA4	T cells	+6230G/G +49A/A	Being related with AIP resistance; marker of risk of relapse in AIP	Umemura et al. [22]
KCNA3	T cells	SNP (rs2840381, rs1058184, rs2640480, rs1319782)	T cell proliferation and activation	Ota et al. [23]
CFTR	—	Variants (1556V, 5T, S42F, etc.)	Predictors of a slow and reduced response to steroid treatment in AIP	Chang et al. [24]

Table 3: Symptoms of AIP in different studies.

Year	Number of patients	Ethnic	Male : female	Jaundice	Abdominal pain	Weight loss	No symptoms
2008 [41]	25	Chinese	22 : 3	18 (72%)	11 (44%)	10 (40%)	3 (12%)
2011 [42]	731	8 countries	—	Type 1 AIP 75% Type 2 AIP 47%	Type 1 AIP 41% Type 2 AIP 68%	—	—
2015 [43]	705	Chinese	4.47 : 1	63.4%	62.3%	45.1%	2.9%
2016 [44]	52	Spain	—	27 (51.9%)	34 (65.4%)	—	—

pathogenesis of AIP. While combining amylase alpha 2A with IgG4 in diagnosing AIP, the specificity can be 99%, higher than the specificity of 96% while using IgG4 only in a clinical study [32].

As for innate immune response, Watanabe et al. reported that activation of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain- (NOD-) like receptors (NLRs) in monocytes and basophils of patients with IgG4 related disease (IgG4-RD) induced IgG4 production by B cells via B cell activating factor (BAFF) [33, 34]. What is more, Fukui et al. reported that abundant infiltration of TLR-7 positive M2 macrophages was observed in the pancreatic tissues in type 1 AIP patients [35].

As for adaptive immune response, B cells and T cells are unavoidable topics. A recent study showed that increased $CD19^+CD24^{high}CD38^{high}$ regulatory B cells (Bregs) might suppress the disease activity of type 1 AIP, while the decreased $CD19^+CD24^{high}CD27^+$ Bregs may be involved in the development of type 1 AIP [36]. Circulatory naïve regulatory T cells (Tregs) are significantly decreased in peripheral blood, while memory T cells are significantly increased in type 1 AIP patients [37]. In addition, prominent infiltration of Tregs with upregulation of IL-10 is observed in the liver of type 1 AIP patients [38]. Li et al. found significant $CD8^+$ T lymphocyte infiltration in the pancreas and extrapancreatic lesions in a case of AIP misdiagnosed as pancreatic cancer, indicating that $CD8^+$ T lymphocyte might have some effect on the cause of AIP [39].

4. Clinical Manifestation

The clinical manifestations of AIP are complex and lack of specificity; therefore, it is extremely difficult to diagnose AIP from symptoms only. Type 1 AIP is typically diagnosed later in life (the mean age at diagnosis is older than 60 years) [2, 18]. Obstructive painless jaundice and upper abdominal pain are the most common complaints. Other rare symptoms include body weight loss, general fatigue, and even no symptoms [40]. A series of studies have been focused on the symptoms and treatments of AIP in different countries and have got different results [41–44] (shown in Table 3). A retrospective study from China showed that the jaundice accounted for 72% and abdominal pain was 44% [41]. Another multicenter study in Spain indicated that abdominal pain accounted for 65.7% and obstructive jaundice was 51.9% in AIP patients [44]. Ueki et al. [45] reveal that type 2 AIP can have the symptoms of acute, constant abdominal pain like in acute pancreatitis, different from the character of chronic pancreatitis.

Besides, AIP can cause extrapancreatic lesions including sclerosing cholangitis, retroperitoneal fibrosis, lachrymal and salivary gland lesions, pulmonary lesions including hilar lymphadenopathy, and tubulointerstitial nephritis, hypophysitis, chronic thyroiditis, and prostatitis [5, 15, 17, 40, 46–50] and biliary tract is the most commonly involved extrapancreatic site [43, 46], which probably explains why there is painless jaundice in AIP patients.

AIP has certain comorbidities. Finkelberg et al. [51] reported that, in AIP patients, approximately 50% have

Table 4: Comparisons of diagnostic criteria in different countries.

Diagnostic criteria	Japanese criteria(2006) [55]	SIHORts (2006) [57]	Korean criteria (2007) [56]	Asian criteria (2008) [58]
A: imaging	Diffuse or segmental narrowing of the MPD; diffuse or localized enlargement of the pancreas	Typical imaging features: diffusely enlarged gland with delayed (rim) enhancement; diffusely irregular and attenuated MPD Atypical imaging features: focal pancreatic mass, focal pancreatic duct stricture	Diffuse enlargement of pancreas and diffuse or segmental irregular narrowing of MPD	Typical imaging features: diffusely enlarged gland with delayed (rim) enhancement; diffusely irregular and attenuated MPD Atypical imaging features: focal pancreatic mass, focal pancreatic duct stricture
B: serology	High serum γ globulin, IgG, IgG4, or the presence of autoantibodies	Elevated serum IgG4 level	Elevated levels of IgG and/or IgG4 or detected autoantibodies	High level of serum IgG or IgG4 or detected autoantibodies
C: histology	Infiltration of lymphocytes and plasma cells	Lymphoplasmacytic infiltrate with storiform fibrosis showing abundant (>10 cells/HPF) IgG4-positive cells	Fibrosis and lymphoplasmacytic infiltration	Lymphoplasmacytic infiltration with fibrosis, with abundant IgG4-positive cell infiltration
D: other organ involvement	Not included	Biliary stricture, parotid/lacrimal gland involvement, mediastinal lymphadenopathy, retroperitoneal fibrosis	Included	Not included
E: steroid effect	Not included	Included	Included	Included
Definite diagnosis	Criterion A + B Criterion A + C	Criterion A + B Criterion A + C Criterion A + D Criterion A + E	Criterion A + B Criterion A + C Criterion A + D Criterion A + E	Criterion A + B Criterion A + C Histology shows the presence of lymphoplasmacytic sclerosing pancreatitis in the resected pancreas

diabetes. More and more attention is focused on the relationship between AIP and inflammatory bowel disease, and the prevalence of IBD in patients with AIP seems to be increased compared to the general population, with 6 to 27% of AIP patients having concomitant IBD [45, 52, 53], especially in type 2 AIP.

5. Diagnosis

5.1. Diagnosis Criteria for AIP. In 2002, the Japan Pancreas Society (JPS) [54] first proposed the diagnostic criteria for AIP and made the image abnormal findings such as irregular narrowing of the main pancreatic duct (MPD) (>one-third of the entire pancreas) and parenchymal swelling as necessity, accompanied with either of the following two: (1) serology showing hypergammaglobulinemia (>2 g/dL, autoantibodies) and serum IgG elevation (>1800 mg/dL) and (2) characteristic pathological findings including lymphoplasmacytic infiltration with fibrosis. The JPS criterion was revised in 2006 [55], and it first proposed the IgG4 elevation as the serology finding, which is important for the diagnosis of AIP even until now. Because of the limitations in JPS, 2006 [55], Korean Kim criteria [56] occurred, including four parts of imaging, laboratory examinations, histology, and steroid effect. Subsequently, HISORt [14, 57] (based on the four parts in Korean criteria, other organ involvement was added), Asian [58] (histology only can be used to diagnose AIP when it meets the demand), and Manheim criteria [59] have been proposed around the world. In 2011, Shimosegawa et al. [5] first proposed the ICDC for AIP, which is the most accepted major diagnostic criterion. Later, JPS 2011 [60, 61] was proposed in response to the ICDC's inclusion of response to steroid treatment. Table 4 shows the comparisons of diagnostic criteria in different countries.

There are several diagnostic criteria for AIP in different countries, but ICDC is the first universally accepted criterion of AIP because it considers ethnic and region differences and classifies AIP into two subtypes. The diagnosis of AIP includes five dimensions: serology, histology, imaging, other organ involvement, and steroid effect.

5.2. Serology Changes in AIP. Since being proposed by JPS 2006, serum IgG4 elevation is widely used in the diagnosis of AIP. However, IgG4 has its limitations. Studies have shown that 4–10% of both healthy controls and controls with other diseases have high serum IgG4 concentrations [62–64]. In addition, about 20% of patients with AIP have normal serum IgG4 concentrations at presentation [63, 65]. A systematic review with meta-analysis about IgG and IgG4 shows that the pooled sensitivity of serum IgG4 was 0.74 and the pooled specificity was 0.94 [66]. An ideal serological marker should be both sensitive and specific, while IgG4 is neither sufficiently sensitive nor specific. Besides, elevated IgG4 is seen only in type 1 AIP whereas type 2 AIP often has normal IgG4 level. Considering these two factors, searching for new serological marker is essential and valuable. Song et al. [67] proposed combining measurement of serum IgG and IgG4 instead of IgG4 alone to increase the sensitivity in diagnosing AIP. Recently, Hao et al. [68] explored that hybrid kappa (κ)/lambda (λ) antibody, which composes a substantial portion of IgG4 in normal human serum and is formed by two IgG4 heavy chains plus one κ and one λ light chain, is a new serological marker for diagnosing AIP. The sensitivity and specificity of hybrid κ/λ antibody were 80.3% and 91%, respectively. While combining serum IgG4 and the hybrid κ/λ antibody, the diagnostic sensitivity could be increased from 78.7% to 90.2% compared with serum IgG4 alone without sacrificing specificity significantly.

5.3. Imaging Features of AIP in Different Examinations. Ultrasound (US) is widely used for its noninvasiveness, low price, and easy operation. US can present the diffuse enlargement and hypoechoic pancreas, but it cannot show the irregular narrowing or stenosis of the pancreatic duct. Quantitative perfusion analysis in pancreatic contrast enhanced ultrasound (DCE-US) can show the vascular lesions of pancreas and play a significant role in differentiating AIP from pancreatic cancer [69].

Computed tomography (CT) is the most important tool to diagnose AIP and distinguish it from pancreatic cancer. The typical image finding is diffuse morphological pancreatic parenchymal enlargement and the atypical findings include focal enlargement of the pancreas, no enlargement or normal pancreas, and mixed patterns [57, 70–73]. AIP demonstrates a diminished pattern of enhancement in the arterial phase and a relatively increased or prolonged enhancement in the delayed or venous phase [72, 74]. And a capsule-like low density rim is a distinctive finding on CT in AIP [72]. However, if there is low density mass on contrast enhanced CT, pancreatic cancer should be considered.

Magnetic resonance imaging (MRI) has advantages over CT on the capsule-like imaging of the pancreatic duct and surrounding lesions, which is the result of the fibrosis of the pancreas. The typical MRI findings include hypointense signal on T1 weighted images and relatively T2 hyperintense signal [75]. Diffusion weighted imaging (DWI) has been increasingly utilized as a MRI sequence for evaluating pancreas [76–78]. Kim et al. [79] found that while perfusion fraction (f) is 0.933, it is most useful for differentiating AIP and normal pancreas and its sensitivity is 85.7% and specificity is 100%. And perfusion fraction (f) and perfusion-related diffusion coefficient (D_{fast}) are more useful than pure molecule diffusion coefficient (D_{slow}) in differentiating pancreatic diseases from normal pancreas.

Magnetic Resonance Cholangiopancreatography (MRCP) is widely used for its advantage of high quality image and noninvasiveness, but for its less sensitivity in the focal form of AIP and pancreatic cancer, it cannot replace ERCP completely. MRCP could show the diffused narrow or segmental stenosis of main pancreatic ducts, the pancreatic segment of common bile duct stricture, proximal bile duct dilation, and gallbladder enlargement [80].Endoscopic retrograde cholangiopancreatography (ERCP) is an invasive method but it is feasible in treatment and diagnosis of AIP and the incidence of ERCP-related adverse events is low in patients with type 1 AIP [81]. Ductal imaging, ERCP, may show a long, narrow ductal stricture, or multiple, noncontinuous strictures without marked upstream dilation, and side branches arising from the stricture [82, 83]. The multicenter study carried out by Sugumar et al. [84] has highlighted that the ability of ERCP to diagnose AIP based on ERCP feature alone is limited, but taken together with clinical symptoms, serology, and/or histology it can be useful.

Endoscopic Ultrasound (EUS) can be utilized to evaluate the pancreatic parenchyma, bile duct, and pancreatic duct, as well as in evaluating the bile duct stricture. The EUS guided fine needle aspiration (EUS-FNA) is not included in ICDC as a method for histopathologic diagnosis of AIP because of the difficulty in obtaining adequate specimens for histological analysis. Although EUS-FNA has its limitations for 20.5% unsuccessful adequate tissue sampling, 23 of the 53 undetermined patients could be diagnosed as definitive type 1 AIP without the aid of pancreatic imaging, serology, other organ involvement, and response to steroids [84], which is unique. The nationwide epidemiology survey of AIP in Japan in 2011 found that the use of EUS-FNA increased to 63.8% from 48.4% and the utility of EUS-FNA for establishing of AIP will be further validated in the future [2, 85–87].

Positron Emission Computed Tomography (PET) can get the total image of every part of the body and it is especially sensitive in finding tumors. PET is more sensitive than conventional imaging to detect organ involvement and uptake of fluorodeoxyglucose in organs other than the pancreas often suggests AIP when the clinical characteristic, histology, and serum detection incline the diagnosis of IgG4 related disease [88, 89].

Every imaging method has its cons and pros (shown in Table 5). What should be emphasized is that methods are not isolated; we can combine two or more methods when needed. Uchida et al. [90] stated that in their institution they initially use CT scans to evaluate the enlarged pancreas followed by evaluation of the main pancreatic duct by ERP. For pancreatic head lesions with obstructive jaundice or biliary enzyme abnormality due to biliary stricture, they first perform diagnostic and therapeutic ERCP. For pancreatic head lesions without obstructive jaundice, they perform

Table 5: Cons and pros of different kinds of imaging.

Imaging	Imaging findings	Advantage	Disadvantage	When to select
US	Diffuse enlargement, hypoechoic pancreas	Low price, noninvasive, and easy to operate	Lack of specificity	Physical examination
CT	Diffuse morphological pancreatic parenchymal enlargement, focal enlargement of the pancreas [72]	Being noninvasive, being easy to operate, high quality image for pancreatic parenchymal enlargement, differentiating AIP from pancreatic cancer	Less sensitivity in the pancreatic and bile duct lesion than MRCP and MRI	Evaluate the pancreatic parenchyma and differentiate AIP from pancreatic cancer
MRI	Hypointense signal on T1 weighted images and relatively T2 hyperintense signal [75]	Being noninvasive, being easy to operate, showing the pancreatic fibrosis	Less sensitivity in pancreatic parenchymal than CT	Evaluate the pancreatic parenchyma
MRCP	Diffused narrow or segmental stenosis of main pancreatic ducts, the pancreatic segment of common bile duct stricture, proximal bile duct dilation, and gallbladder enlargement [80]	Being noninvasive, being easy to operate, presenting the pancreatic duct and bile duct and their relationship	Less sensitivity in the focal lesion of pancreatic parenchymal than CT	Evaluate the bile duct, pancreatic duct, and bile duct stricture
ERCP	Diffuse, irregular narrowing of the MPD [82, 83]	Diagnosis and treatment simultaneously, especially in the case of jaundice	Invasive	Evaluating the bile duct, pancreatic duct, and bile duct stricture, treatment for jaundice
EUS-FNA	—	Get the tissue with much less wound than surgery	Invasive May not get adequate tissue	Get the pancreatic tissue sample
PET	Uptake of fluorodeoxyglucose in organs other than the pancreas [88, 89]	Other organ involvement is easily detected	Expensive	Assess the other organ involvement, exclude malignant tumor

EUS-FNA followed by diagnostic and therapeutic ERCP. For pancreatic body or tail lesions, they first perform EUS-FNA.

6. Differential Diagnosis and the Strategy for Distinguishing AIP from Pancreatic Cancer

As a new and relatively rare pancreatic lesion, AIP is easy to be neglected and misdiagnosed as pancreatic cancer for its clinical and imaging features. As is proposed in ICDC, IgG4 elevation is a high-specific serum marker for AIP [49, 50]. However, Ngwa et al. [91] reported that 10.1% of 548 patients with pancreatic cancer have elevated serum IgG4, which may be confusing when serum IgG4 is used to differentiate pancreatic cancer and AIP. Serum CA19-9 was stated to be useful for distinguishing AIP from pancreatic cancer [92], while CA19-9 can also be elevated in other pancreatic diseases or in other pathological states [93]. Thus, so far, a simple serological marker for the differential diagnosis of AIP from pancreatic cancer is still lacking. What is worse, the differentiation by imaging also presents some problems, especially pancreatic cancer and the focal AIP exhibiting mass formation [7, 8]. Thus, a thorough work-up is essential before either surgery or steroid treatment is planned.

Here we present the American diagnostic strategy to differentiate AIP from pancreatic cancer. In patients with obstructive jaundice and/or pancreatic mass CT findings typical for AIP, the presence of any collateral evidence for AIP (elevated IgG4 or autoantibodies or other organ involvement) is sufficient to make the diagnosis. On the other hand, those with any of the features highly suggestive of pancreatic cancer should generally be managed as cancer unless there is clear evidence of other organ involvement suggestive of AIP. Patients without typical findings of AIP, including those with indeterminate CT findings, should undergo work-up for cancer. If negative, additional collateral evidence for AIP (serum IgG4) should be sought. Diagnosis of AIP is confirmed by pancreatic core biopsy, steroid trial and surgical resection [14] (Figure 1).

7. Treatment for AIP

Glucocorticoids are the routine drug for AIP and rapid response to steroid treatment is one of the primary characteristics of AIP. A poor response to steroid therapy might suggest misdiagnosis, especially in the case of pancreatic cancer. Hart et al. [18] conducted a multicenter, international analysis (1064 patients), showing that 99% of type 1 AIP and 92% of type 2 AIP got clinical remission after steroid treatment. Before induction of remission by an initial steroid therapy, management of blood glucose and biliary drainage is recommended in patients with diabetes mellitus and obstructive jaundice. Generally, patients are given initial oral prednisolone dose of 0.6 mg/Kg/day for induction of remission, which is administered for 2 to 4 weeks. The dose is then tapered by 5 mg every 1 to 2 weeks to a maintenance dose (2.5–5 mg/day) that should be continued for three years as

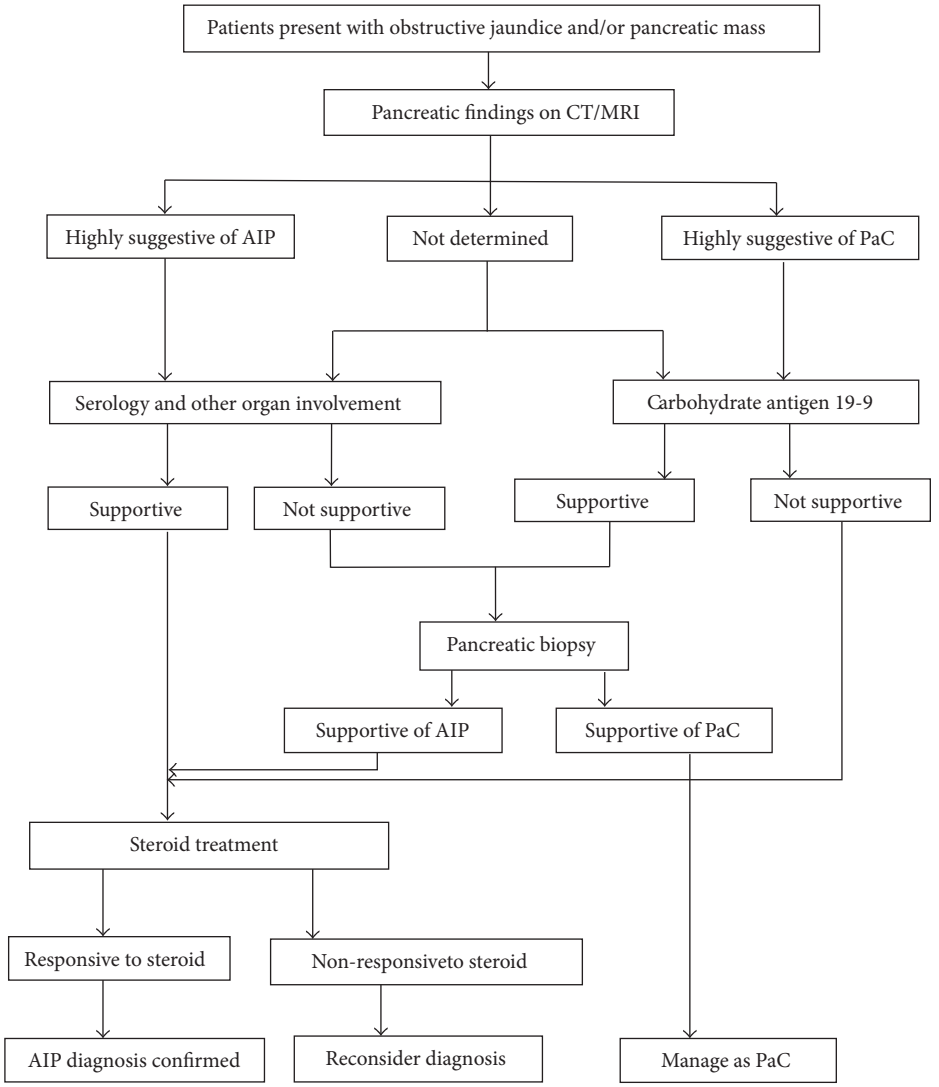

Figure 1: Strategy for distinguishing AIP from pancreatic cancer [14]. CT: computed tomography; MRI: magnetic resonance imaging; PaC: pancreatic cancer; OOI/O: other organ involvement; S: serology; CA19-9: carbohydrate antigen 19-9.

maintenance therapy in Japan [94] and the regimen is utilized in most Asian countries. In a multicenter study in Japan, Kamisawa et al. [95] reported that relapse occurred significantly less during maintenance steroid therapy than after the discontinuation of therapy (23% versus 34%, $P < 0.05$), while in European and American countries, maintenance therapy is not commonly used. Ghazale et al. [96] conducted a study and the initial steroid regimen was as follows: prednisolone 40 mg/day orally for 4 weeks, then tapering by 5 mg/week until 11 weeks, and then having a maintenance dose from 12 weeks (Table 6). As a result, 16 (53%) of 30 patients associated with sclerosing cholangitis relapsed during a median follow-up of 29.5 months. Moreover, long time maintenance steroid therapy may cause steroid-related side effects and not all patients can tolerate them. Therefore, whether a maintenance therapy is needed or not needs international discussion.

In a multicenter study in Japan, the cumulative rate of relapse after starting steroid therapy was 56% at 1 year, 76% at two years, and 92% after 3 years [95]. Hart et al. [18] found the relapse was related to IgG4 related sclerosing cholangitis, no business with serum IgG4 level or pancreatic parenchyma involvement (diffuse or focal pancreatic parenchyma enlargement), while Shimizu et al. [97] confirmed that the rate of decrease in serum IgG4 level was significantly higher in nonrelapse group than in the relapse group after steroid treatment and it might be a predictor of a relapse of AIP. The treatment for relapse is restarting steroids. Whether or not to use alternative immunosuppressant, such as azathioprine, methotrexate, and mycophenolate mofetil [5, 18], depends on the patient's reaction to re-steroid therapy and his tolerance to steroid. Unfortunately, in some cases, the patients cannot tolerate both steroid and immunosuppressant and require drug discontinuation. Rituximab, a monoclonal CD20 antibody, has been shown to be useful in treating AIP patients [98, 99]. The effectiveness of rituximab shed light on the role of B cells in the pathogenesis of AIP because of the B cell depletion. As

Table 6: Management strategy of AIP based on immunology therapy.

Time	0–12 weeks	12 weeks–6 months	6 months–3 years
Japan and Asian countries [94]			
Objective	Induction of remission	Maintenance therapy	
Drug	Prednisolone	Prednisolone	
Dose	0.6 mg/Kg/day for 2–4 weeks, tapered by 5 mg every 1-2 weeks to a maintenance dose	2.5–5.0 mg/day	
American and European countries [19]			
Objective	Induction of remission	Maintenance therapy	Observation
Drug	Prednisolone	Prednisolone	Immunomodulator/rituximab (when relapsing)
Dose	30–40 mg/day for 2–4 weeks, tapered by 5 mg every 1-2 weeks to a maintenance dose	5.0–7.5 mg/day	Undetermined

rituximab is the only drug for induction of remission other than glucocorticoids, it would be extremely useful in patients who are unable to tolerate high-dose corticosteroids, require high doses of prednisolone to maintain remission, or have failed to respond to immunomodulator therapy. Currently approved for treating B cell lymphoma and rheumatoid arthritis, rituximab's approval for treating AIP will come true.

The prognosis of AIP is good in general and the long-term complication is rare. Pancreatic duct stones and canceration are the main sequelae [18]. Kanno et al. [2] conducted the nationwide epidemiological survey in Japan in 2011 and found that during the course of observation (1623.3 days), malignant tumors were detected in 109 of 923 patients (11.2%). Shiokawa et al. [100] reported that AIP patients had a high risk of having various cancers, while Hart et al. [101] reported that cancer risk of AIP patients was similar to that of control subjects. Whether AIP is the risk factor for developing cancer needs further investigation.

8. Conclusion

In conclusion, AIP is a special type of chronic pancreatitis, whose pathogenic mechanism, maybe a combination of genetic factors and immunity abnormality, needs more work to be clarified. The diagnosis of AIP depends on serology, imaging, histology, other organ involvement, and reaction to steroids, while high sensitive serum biomarkers for AIP subtypes lack. AIP reacts well to steroids, but controversy exists on the steroid maintenance and treatment for relapse. For AIP shares similarity with pancreatic cancer in clinical and imaging characteristics, general work-up is necessary to differentiate them. Future research may be focused on the pathogenesis, the novel serum biomarker, and the relapse treatment for AIP.

Competing Interests

The authors declare that they have no competing interests.

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Review Article

The Immunological Basis of Inflammatory Bowel Disease

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Inflammatory bowel diseases (IBDs) are chronic ailments, Crohn's disease and ulcerative colitis being the most important. These diseases present an inflammatory profile and they differ according to pathophysiology, the affected area in the gastrointestinal tract, and the depth of the inflammation in the intestinal wall. The immune characteristics of IBD arise from abnormal responses of the innate and adaptive immune system. The number of Th17 cells increases in the peripheral blood of IBD patients, while Treg cells decrease, suggesting that the Th17/Treg proportion plays an important role in the development and maintenance of inflammation. The purpose of this review was to determine the current state of knowledge on the immunological basis of IBD. Many studies have shown the need for further explanation of the development and maintenance of the inflammatory process.

1. Introduction

Inflammatory bowel diseases (IBDs), notably Crohn's disease (CD) and ulcerative colitis (UC), are widely considered multifactorial diseases and are characterized by chronic intestinal inflammation [1]. These diseases vary according to the affected gastrointestinal area, the depth of the inflammation in the intestinal wall, and the peculiarity of their pathophysiology. The prevalence of IBD is highest in the second to third decade of life with another peak in the 60–70-year-old group [2]. At the onset and during the progression of the disease, associations occur among the genetic factors (which predispose the patient to develop the disease), the environmental factors (which modulate the inflammatory pathways), and the composition of the microbiota [3].

Crohn's disease (CD) is a chronic, transmural, and segmental inflammatory disease. It may affect any part of the gastrointestinal tract, from the mouth to the anus, but is located usually in the terminal ileum. It is characterized by the formation of ulcers, fistulas, stenosis, and intestinal granulomas, with periods of aggravation and remission. Several additional intestinal manifestations may be observed [4]. Ulcerative colitis (UC) is also a chronic inflammatory disease. However, it can affect only the mucosa of the colon and the rectum [5].

The clinical characteristics of IBD are hemorrhagic diarrhea, abdominal pain, tenesmus, urgency to evacuate, anorexia, and weight loss [5, 6]. The etiopathology is not well understood, but environmental factors may be involved, as they predispose genetically susceptible individuals. The severity of the symptoms varies from mild to severe, especially in those who do not respond to the treatment. Patients who do not respond to clinical management and have complications of the disease usually require surgical intervention [7]. The pathophysiology of IBD is not well understood, but there are several hypotheses about its origin: impaired mucosal barrier; dysbiosis; persistent pathogenic infection; and immune deregulation.

2. Mucosal Barrier

Patients with genetic susceptibility to IBD are exposed to environmental factors, such as diet and lifestyle, which can induce immune responses that impair the mucosal barrier. The integrity of the epithelial layer enables the intestinal lumen bacteria to communicate with the immune system [8].

The first physical barrier on the mucosal surface is the mucous layer. It is formed by inner and outer layers that are produced by the polymerization of gel-forming mucins secreted by Goblet cells [9]. The inner layer is sterile and the

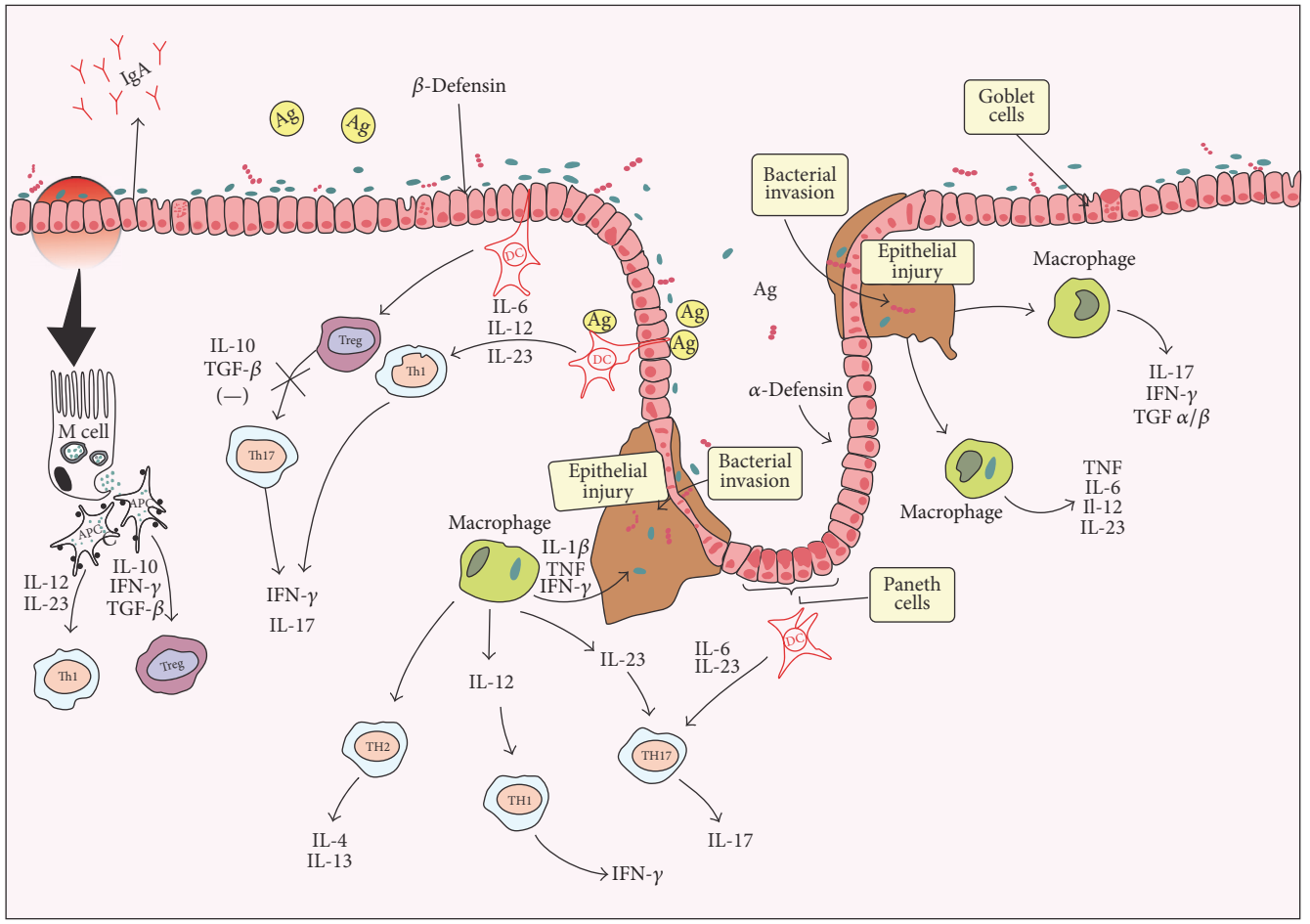

Figure 1: Intestinal epithelial barrier and the immune system in inflammatory bowel disease. Ag: antigen; APC: antigen presenting cells; IL: interleukin; IFN-γ: interferon gamma; IgA: immunoglobulin A; M cell: microfold cell; TGF-β: transforming growth factor beta; TGF-α: transforming growth factor-alpha; Th: T helper cell; Treg: regulatory T cells; TNF: tumor necrosis factor.

outer is inhabited by commensal bacteria that consume the nutrients in the mucin glycan [9].

The intestinal epithelium is the next barrier and it is considered the second line of defense against bacterial invasion. It comprises enterocytes and specialized epithelial cells called Goblet and Paneth cells [9]. Intestinal epithelial cells (IECs) play a key role in the mucosal barrier, as they prevent the influx of antigens and the invasion by both pathogens and commensal microorganisms [8]. They play a pivotal role in the maintenance of tolerance toward alimentary antigens and commensal microbiota and also activate both innate and adaptive immune responses [10] (Figure 1).

To protect the mucosal barrier, the IECs present tight junctions and produce mucins and defensins (α-defensins are produced by Paneth cells and β-defensins are produced by most of the IECs). IECs also express toll-like receptors (TLR) and nucleotide oligomerization domain receptors (NOD), which are pathogen-sensitive innate immune receptors. IECs then produce chemokines and cytokines to recruit immune cells [8]. Therefore, TLR signaling pathways produce proinflammatory cytokines, such as interleukin- (IL-) 12 and IL-6 by IECs, besides helping to keep the epithelial barrier intact [8, 11]. An impaired epithelial barrier leads to an increased intestinal permeability, which has been observed in CD and also in UC [12]. Some Genome-Wide Association Study (GWAS) suggests that it might represent a primary pathogenetic mechanism in IBD [9]. TLRs belong to the class of transmembrane receptors, called pattern recognition receptors (PRRs), acting as a pro/anti-inflammatory gene activation inducers and control the adaptive immune responses [13, 14]. The TLR family comprises ten different transmembrane receptors that may be found in two locations: in the cell membranes, as is the case with TLR1, TLR2, TLR4, TLR5, and TLR6; into intracellular compartments, such as TLR3, TLR7, TLR8, and TLR9. These genes can be expressed constitutively or inductively along the gastrointestinal tract and in various cell types including enterocytes, Paneth cells, enteroendocrine cells, Goblet cells, myofibroblasts, and subepithelial cells of the intestine immune system, such as monocytes, macrophages, dendritic cells (DC), and CD4 + [15, 16]. In healthy individuals, TLR2 and TLR4 receptors are expressed in smaller amount compared to CD patients, as what triggers a faulty recognition. Environmental, genetic, and immunological factors may alter those receptors [15].

TLR4 is responsible for the recognition of lipopolysaccharide (LPS) and its immune response. The LPS signaling pathway triggers changes in an immunological response, which increases intestinal inflammation [17]. To prevent improper activation against commensal microbiota, TLR is inhibited by cellular mechanisms in the intestinal mucosa. When there is contamination by pathogenic bacteria, the inhibiting TLR mechanism is disabled and positive regulators allow TLR signaling favoring the immune response and the elimination of pathogens [15]. However, the hyperactivation of TLR causes chronic inflammation in IBD. The TLR4 has a significant increase in IEC and in primary mononuclear cells (LPMNCs) of *lamina propria* throughout the lower gastrointestinal tract in IBD patients, which shows the role of this receptor on the mucosal inflammation [15] (Figure 2).

3. Microbiota

Although no single agent has been proven to cause IBD, a role for gut microbes has been suspected since the early descriptions of potential infectious pathogens [18]. IBD is clearly associated with intestinal dysbiosis, which is the imbalance in the functions of gut microorganisms that impair host-microbe and immune homeostasis [18]. Human gut contains about 10^{11}-10^{12} microorganisms per gram of intestinal lumen content. These microorganisms, called commensal bacteria, can be beneficial to the organism in normal circumstances, as they help to protect the intestinal epithelium [19, 20]. Most of them represent two different phyla, which are the majority of gram-negative bacteria (such as Bacteroidetes) and gram-positive bacteria (such as Firmicutes); the remainder represent a rarer phyla such as Proteobacteria (*Escherichia* and *Helicobacter*) and Actinobacteria; they also include fungi, protists, and viruses [21].

Patients with genetic susceptibility are exposed to environmental factors, such as diet and lifestyle, which can induce immune responses that alter the intestinal microbiota and impair the mucosal barrier [8, 22, 23]. Devkota et al. [23] demonstrated that a diet that does not change the intestinal microbiota is critical to the prevention of IBD. An increase in the incidence of UC was observed in IL-10 deficient mice fed with high levels of saturated fat. This diet promoted the growth of *Bilophila wadsworthia*, a commensal bacterium. This proliferation was probably due to the changes in the composition of bile acid caused by high intake of saturated fat, leading to dysbiosis. von Mutius [24] suggested that the exposure to commensal bacteria during childhood is associated to protection against the development of IBD, for it is critical to stabilize immune tolerance.

In IBD, a dysfunctional interaction between gut microbiota and the mucosal immune system takes place, which may lead to the loss of intestinal immune tolerance by an overreaction of effector T cells that react against common microbial antigens. Thus, there is a decrease of Treg cells that do not properly modulate the effector T cell. This triggers changes in the type and number of microorganisms in the intestinal mucosa, which ultimately leads to an inadequate immune response [25]. Some mouse studies have shown more clearly that the enteric microbiota regulates the development of intestinal immune cell [8]. The balance of some factors, such as TGF-β and IL-6, plays a key role in the differentiation of Th17 and Treg [26, 27]. Commensal bacteria can regulate the development of both Th17 and Treg cells suggesting the relevance of local environment induced by commensal microorganisms in immunological homeostasis of gut-associated lymphoid tissues (GALT) [8]. Some other studies highlighted the importance of commensal bacteria for Th17 differentiation in both health and disease: Atarashi et al. [28] demonstrated that commensal bacteria-derived adenosine 5′-triphosphate (ATP) activates a specific subset of colonic *lamina propria* cells, defined as $CD70^{high}CD11c^{low}$ DCs, which leads to Th17 cells differentiation. In response to ATP stimulation, this subset expresses Th17-prone molecules, such as IL-6 and IL-23p19, and induces Th17 differentiation of cocultured naive CD4+ T cells. Ivanov et al. [29] reported that a small commensal intestinal microbiota, segmented filamentous bacterium (SFB), is sufficient to induce Th17 cells in the intestinal *lamina propria*.

One of the many mechanisms that affects host inflammatory responses is associated with short-chain fatty acids (SCFA). Their levels are significantly decreased in IBD; it may be a key factor compromising both intestinal and immune homeostasis [30]. Atarashi et al. [28] demonstrated that SCFA-producing bacterial strains in *Clostridia* clusters IV, XIVa, and XVII from a healthy human fecal sample induced colonic regulatory T (Treg) cell differentiation, its expansion, and function.

In IBD, B cell responses also occur: IgA is a major class of immunoglobulin produced in the mucosa, including the gut. In the intestinal lumen, IgA is produced as polymeric IgA at high concentrations, which is transported by the polymeric immunoglobulin receptor (pIgR) expressed on IECs and released into the intestinal lumen as secreted IgA (SIgA). SIgA covers antigens in order to inhibit their binding to the host epithelium and, therefore, the penetration into the *lamina propria* [31, 32]. The binding of IgA to the commensal *Bacteroides thetaiotaomicron* inhibits innate immune responses by impairing bacterial gene expression [33].

Mononuclear phagocytes, such as macrophages and DCs, are responsible for the lack of immunological response to commensal bacteria, which is relevant to maintaining gut homeostasis [31, 32]. The microbiota is important for the production of pro-IL-1β and the precursor of IL-1β, in resident mononuclear phagocytes. When the epithelial barrier is intact, commensal bacteria cannot induce the maturation of pro-IL-1β into biologically active mature IL-1β and thus a state of low response is maintained [32]. By contrast, enteric pathogens, such as *Pseudomonas aeruginosa* and *S. Typhimurium*, may induce the maturation of pro-IL-1β as it activates caspase-1 via the NLRC4 (NOD-, LRR-, and CARD-containing 4) [32]. Microbiota also promotes immune response by the production of IL-22 by innate lymphoid cells (ILCs) [34]. A study with germ-free mice reported an impaired gut IL-22 production, suggesting that there may be a requirement for commensal bacteria or their metabolites [35]. Mice with impaired cells that express

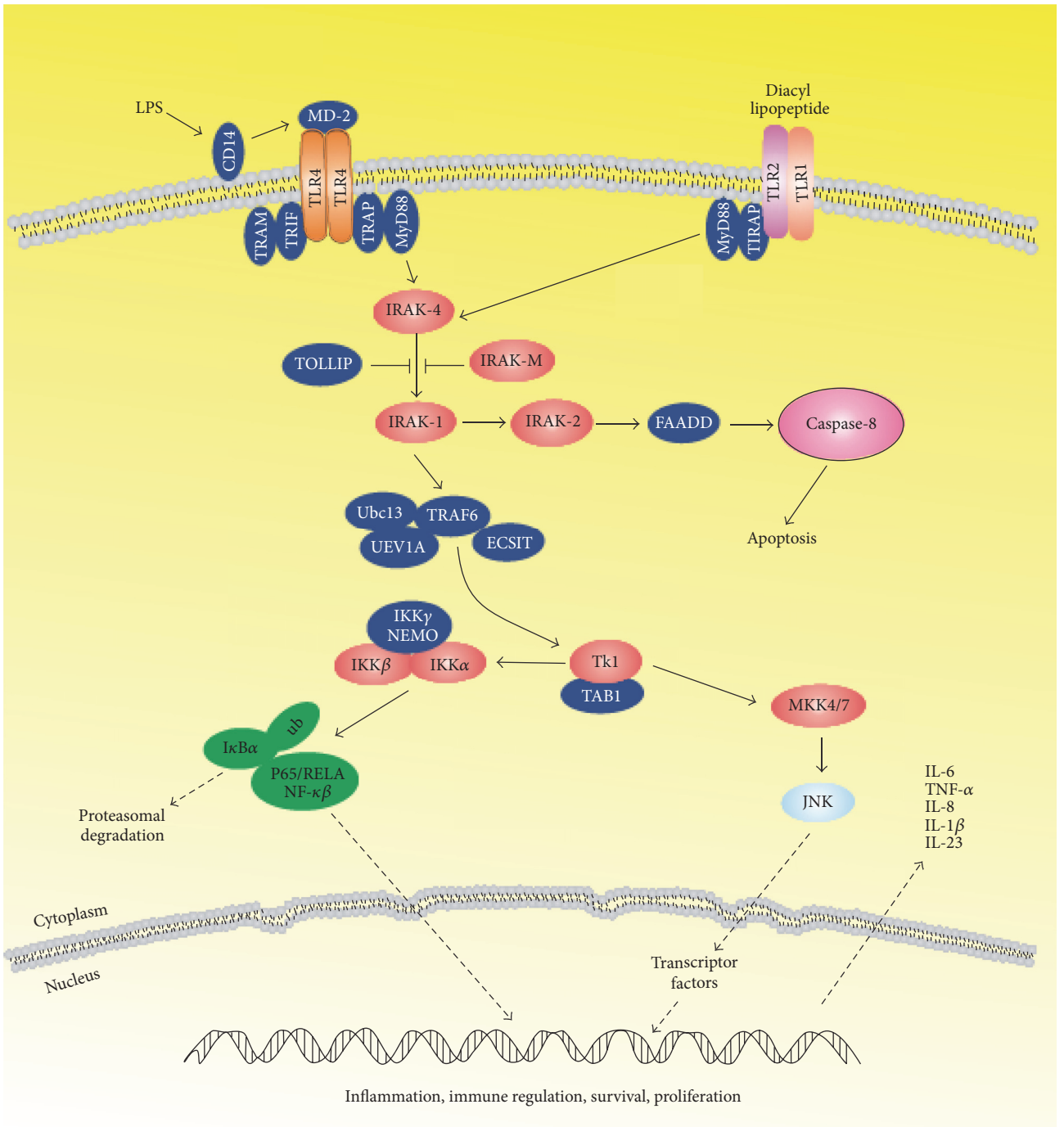

Figure 2: Toll-like receptor signaling pathways. LPS: lipopolysaccharide; CD14: cluster of differentiation 14; MD-2: lymphocyte antigen 96; TLR: toll-like receptor; TRIF: TIR domain-containing adaptor-inducing IFN-β; TRAM: TRIF-related adaptor molecule; TRAP: tartrate-resistant acid phosphatase; MyD88: myeloid differentiation primary response 88; IRAK4: interleukin-1 receptor-associated kinase 4; IRAKM: interleukin-1 receptor-associated kinase M; IRAK1: interleukin-1 receptor-associated kinase 1; IRAK2: interleukin-1 receptor-associated kinase 2; TOLLIP: toll interacting protein; FADD: Fas-associated protein with death domain; Caspase-8: cysteine-aspartic protease 8; TIRAP: toll-interleukin-1 receptor domain-containing adaptor protein; UBC13: ubiquitin-conjugating enzyme; TRAF6: TNF receptor-associated factor 6; UEV1A: ubiquitin-conjugating enzyme E2 variant 1A; ECSIT: evolutionarily conserved signaling intermediate In toll pathway; IKKγ: nuclear factor kappa-B kinase subunit gamma; IKKβ: nuclear factor kappa-B kinase subunit beta; NEMO: NF-kappa-B essential modulator; IKKα: nuclear factor kappa-B kinase subunit alpha; TK1: thymidine kinase 1; TAB1: TGF-beta activated kinase 1; MKK4/7: mitogen-activated protein kinase kinases 4; JNK: Janus kinase; ub: ubiquinization; ICBα: inhibitor of kappa-B; p65/RELA: nuclear factor NF-kappa-B P65 subunit; NF-kB: nuclear factor kappa-B; IL: interleukin; TNF-α: tumor necrosis factor-alpha.

Table 1: Main cytokines of the innate immune response, cells that produce them, and the principle actions.

Cytokines	Types of cells	Main functions in IBD
IL-1	Monocytes Epithelial cells Macrophages Endothelial cells	Activating T cells to produce IL-8 and IL-6 Development of IBD [47–49]
IL-6	Macrophages Endothelial cells Fibroblasts	Playing a key role in the differentiation of Th17 and Treg cells, in balance with some factors, such as TGF-β [26, 27, 49]
IL-12	Macrophages Dendritic cells	Promoting the differentiation of Th1 cells [61]
IL-23	Macrophages	Stimulating the production of IL-17, TNF-α, and IL-6 [61]
TNF-α	Macrophages Dendritic cells Endothelial cells	Acting on Th2 surface receptor promoting the proliferation of this cell type [49] Inhibiting Treg cells [52–54]

IL-22 showed an increase in the susceptibility to infection by *C. rodentium*, which suggests that commensal bacterial-driven IL-22 produced by ILC3s is important for protection against infectious pathogens [34, 36, 37].

4. Innate Immunity

Innate immunity is the first defense against invading microorganisms and other harmful agents. Innate response is activated minutes after the invasion by microorganisms. It may last a few hours and has no immunological memory [38]. The tissues affected by IBD present activated macrophages, which also express the CD14 monocyte marker (cluster differentiation 14) and they are phenotypically heterogeneous, unlike what is observed in the normal gut.

Macrophage cells can eliminate specific pathogens, such as peptides and lipopolysaccharides using free radicals and proteases. Cell membrane histocompatibility complex is responsible for specific pathogen-associated antigen. After formation of this complex, T cells are presented to the antigens located on the surface receptors [39]. During an IBD acute phase, the number of macrophages in the intestinal mucosa increases dramatically. In this process, macrophages express large number of T cells and costimulatory molecules such as CD40, CD80, and CD86, involved in the inflammatory process.

In nonpathogenic conditions, macrophages are limited by the intestinal mucosal microenvironment. They present non-inflammatory phenotypes that are decoded by a decreased expression of receptors related to innate immunity activation. Therefore, a limited production of proinflammatory cytokines, such as interleukin- (IL-) 1α and IL-1β, and tumor necrosis factor-alpha (TNF-α) is observed [40, 41].

Another cell type involved in this process is dendritic cells (DC), which are antigen presenting cells (APC). They are directly related to local immune regulation. In both CD and UC, DCs are activated in small numbers but have strongly expressed microbial receptors. This causes an overexpression of some proinflammatory cytokines, such as IL-6 and IL-12 [42]. DCs transport antigens to the gut-associated lymphoid tissue (GALT) where the naive T cells are activated. They can determine whether there will be an immune response or not. Due to TLR, DCs can recognize certain molecular structures of the bacteria, such as the PAMP (pathogen-associated molecular pattern), and it enables them to distinguish very similar microorganisms. Because of these functions, DCs became fundamental in IBD, as they are responsible for the balance between the tolerance to commensal microorganisms and immune activity [43].

In healthy patients, TLR signaling helps to protect the epithelial barrier and assists tolerance to commensal bacteria. However, malfunction in TLR signaling can induce an intestinal inflammatory response with different clinical phenotypes, including the IBD [43]. A major target of the TLR signaling is the activation of transcription factor NF-kB [44], which regulates the expression of a variety of genes responsible for controlling the innate response, such as IL-1, IL-2, IL-6, IL-12, and TNF-α [45, 46]. Both IL-1 and TNF-α share numerous proinflammatory property responsible for the development of IBD [47, 48]. Table 1 shows the main cytokines involved in innate immune response.

5. Adaptive Immunity

Adaptive immunity presents an important role in the pathogenesis of the disease. T cells regulate the immune response in IBD. They proliferate in the peripheral blood and differentiate when they are stimulated by the presence of antigens. The main subtypes of T helper (Th) cells are Th1, Th2, Treg, and Th17. Each of these subtypes has relevant immune functions. For example, Th1 eliminates pathogenic agent present in the cells; Th2 controls allergic reactions and protects the body from parasites; Th17 among all its functions are to remove the extracellular bacteria and fungi; Treg cells are to promote tissue repair. However, alterations in the proliferation of T cells and their subsets may have an excessive increase of chemokines and cytokines, leading to the worsening or maintenance of the mucosal inflammatory process [49].

After the identification of antigens in gut-associated lymphoid tissue (GALT), the activation of effector CD4+ and CD8+ T cells (Th1 and Th2) occurs, as well as the maturation of B lymphocytes that produce antigen-specific

immunoglobulins. T cells in contact with IFN-γ differentiate into Th1 cells. Th1 cells are responsible for secrete different types of proinflammatory cytokines, such as IL-1, IL-2, IL-6, IL-8, IL-12, TNF-α, and IFN-γ [49]. IFN-γ is responsible for macrophage activation. Studies in mouse models in which CD was induced by trinitrobenzene sulfonic acid and IFN-γ expression were increased in the local intestinal mucosa and in the spleen [50]. The antigen presenting cells secreting IL-4 act on the Th cells surface receptors activating STAT-6, which promotes the differentiation into Th2 cells [49]. IL-13 and TNF-α act on Th2 surface receptor activating and promoting the proliferation of this cell type [49]. The increase of Th2 is simultaneous to the increase of IL-5 and IL-13 in the UC inflamed mucosa [51]. Th2 cells secrete IL-4, IL-5, IL-9, and IL-13, which regulate the differentiation and activation of B cells [52–54]. These two cell types also secrete TNF-α, a Treg suppressor. However, Th1 cells secrete them in higher amounts. Breese et al. [55] observed that there is a higher increase of secretion of IFN-γ in CD than in UC, and Fuss et al. [56] observed a higher expression of IL-5 in UC than in CD. Therefore, Th1 and Th2 cells are essential in the development of intestinal inflammation. This response is firstly induced by IL-12 produced from active DC and is mediated by an excessive IFN-γ production [57, 58]. The balance between Th1 and Th2 occurs when the released cytokines inhibits the action of another Th cell, as for example, IFN-γ secreted by Th1 cell inhibits proliferation of Th2 cells, while IL-4, IL-10, and IL-13 secreted by Th2 cells inhibit exacerbated responses of Th1 cell [49]. Thus, the imbalance of Th1/Th2 subsets is directly involved in the pathogenesis of several autoimmune and immune-mediated diseases and inflammatory diseases, and they have fundamental performance in the development and maintenance of inflammation in IBD [59]. Proinflammatory cytokines as IL-1, IL-2, IL-6, and IL-8, which are secreted by Th1, are associated with cellular immune responses and anti-inflammatory cytokines as IL-4, IL-10, and IL-13, which are secreted by Th2 cells, are directly involved in humoral immune response. The balance between proinflammatory and anti-inflammatory properties is determined by the Th1/Th2 cells ratio, determining the types of immune responses that patients develop [49]. Therefore, many researchers have been studied cytokines and T cells subtypes to discover new targets for the IBD treatment [60].

Moreover, APCs produce IL-12, which induces the expression of IFN-γ by Th1 cells, besides IL-2 and TNF-α. Th2 cells produce IL-4, which stimulates the production of IL-5 and IL-10 [61]. Th1 cells increase the expression of MHC-II molecules (Major Histocompatibility Complex II) in the APC, which activates CD8+ T cells and macrophages [62]. The progression of CD is mainly mediated by CD4+ Th1 and Th17 cells, and IFN-γ is one of the main cytokine expressed in this disease [51]. The antigen presentation mediated by MHC-II is fundamental to develop a CD4+ T cell immune response [63]. The MHC-II molecule is primarily expressed on mature APCs, which leads to the activation of effector T cell and FoxP3+ Treg cell [64]. Due to MHC-II antigen presentation machinery, IECs are able to process and present intestinal luminal antigens [65]. Thelemann et al. [63] reported that mice with MHC class II depletion specifically in IECs have increased innate immune cell infiltration and proinflammatory cytokines. Besides, they presented Th1 response with similar levels of Th17 cells compared to wild littermates. In contrast, mice presenting MHC class II depletion in innate lymphoid cells type 3 (ILC3s) have increased Th17 cell numbers compared to control group [63]. The results of these studies suggest that ILC3s limit Th17 differentiation through the expression of MHC-II by an unknown mechanism and highlight the multiple capable of cell type's antigen presentation and T cell differentiation [66, 67].

In the immunological responses described above, one that stands out in the CD development process is the activation of IL-23/IL-17 response in the target tissues [69], in addition to the Th1 response. IL-23 is produced by APC, DC, and macrophages, and it stimulates the production of IL-17, TNF-α, and IL-6 by Th17 cells [61]. IL-17 presents a proinflammatory activity, which induces the production of cytokines that increase Th1 response; chemokine expression; adhesion molecules by epithelial and endothelial cells; fibroblast proliferation; and growth factor expression, such as G-CSF (Granulocyte Colony Stimulating Factor) and GM-CSF (Granulocyte Colony Macrophage Stimulating Factor) [68]. Table 2 shows the main cytokines involved in adaptive immune response.

Humoral immunity is also changed, and B cells produce and secrete a deregulated amount of antibodies, especially IgG, IgM, and IgA [70]. In CD, the IgG-1, IgG-2, and IgG-3 levels are high both in serum and in the intestinal mucosa, compared to healthy subjects [71]. Several autoantibodies and antibodies against specific microorganisms were identified in IBD [72]. The best known are the neutrophil cytoplasmic antibody (ANCA) and the antibody against *Saccharomyces cerevisiae* (ASCA). ANCA autoantibody production is triggered by bacterial antigens. It is present in 65 to 70% of patients with UC and constitutes one of the few markers for the disease, as the other antibodies are more efficient markers for CD. The ASCA antibody is positive in 55 to 70% of CD patients. Other antibodies are OmpC, I2, CBir1-flagellin, A4-Fla2 flagellin and Fla-X. OmpC originates from an antigen of the membrane surface proteins of the bacteria *E. coli*. In contrast, I2 reacts against *P. aeruginosa*, while CBir1-flagellin antibody is directed against flagella of commensal bacteria [73, 74]. The A4Fla2 and Fla-X flagellins have been recently discovered and some CD patients are seropositive. In a prospective study evaluating 252 patients with CD, 59% were positive for A4-Fla2 and 57% for Fla-X, while 76% of the overall sample had localized disease in the small intestine [75]. Another study showed that patients undergoing ileal pouch anal anastomosis for UC with positive ASCA IgG and CBir-1 were related to the development of fistulas and CD in the ileal pouch. The identification of this group of patients with a high risk of complications may allow early and more aggressive measures to prevent ileal pouch failure [76].

More recently, studies have evaluated anti-glycan antibodies, which act against saccharide components of the cell membrane of microorganisms (bacteria, fungi and viruses). These antibodies are found in a variable percentage of patients with CD (10–28%, except for g-ASCA,

Table 2: Main cytokines of the adaptive immune response, cells that produce them, and the principle actions.

Cytokines	Types of cells	Main functions in IBD
IL-2	T cells	Inducing proliferation of T and B cells and the production of IFN-γ [49]
IL-4	Th2 cells Mast cells	Promoting the differentiation of Th2 cells Inhibiting exacerbated responses of Th1 cells [49]
IL-10	Macrophages Dendritic cells Treg	Inhibiting exacerbated responses of Th1 cell [49]
IL-17	Th17 cells Neutrophils	Promoting inflammation by inducing the production of IL-6, IL-1, and TNF-α Inducing the production of cytokines that increase Th1 response Inducing chemokine expression, adhesion molecules by epithelial and endothelial cells, fibroblast proliferation, and growth factors expression, such as G-CSF and GM-CSF [68]
TGF-β	T cells Macrophages Fibroblasts	Inhibiting Th subtypes, such as Th1, Th2, and Th17 cells [49] Playing a key role in the differentiation of Th17 and Treg, in balance with some factors, such as IL-6 [26, 27]
IFN-γ	Th1 cells TCD8$^+$ cells NK cells	Activation of macrophages [50] Inducing the production of IL-12 [57, 58]

whose sensitivity is higher, 46–60%). The most well-known antibodies are: anti-*Saccharomyces cerevisiae* antibody (gASCA), anti-laminaribioside carbohydrate antibody (ALCA), anti-chitobioside carbohydrate antibody (ACCA), anti-mannobioside carbohydrate antibody (AMCA), anti-laminarin IgA (anti-L), and anti-chitin IgA (anti-C). Besides aiding in the diagnosis of CD, these markers may predict disease progression. For example, gASCA and AMCA are signs of short duration disease. gASCA and ALCA are biomarkers of disease at a young age, and ACCA suggests long-term illness, while anti-L and anti-C indicate colonic involvement. Although the sensitivity is not high for all these markers, the specificity is slightly higher (about 40%) [77]. The findings on anti-glycan antibodies suggest a connection between the innate and adaptive immune systems. This reflects the loss of tolerance to commensal microorganisms, which is considered a hallmark of the immunopathogenic process in IBD.

6. T Regulatory Cells

Treg cells are cells capable of inhibiting other Th subtypes, such as Th1, Th2, and Th17 through the release of cytokines IL-10 and TGF-β and by direct contact with the surface of Th cell [49]. Tregs have as their main characteristic a specific surface marker called Foxp3, which distinguishes it from other Th subtypes. These cells are subdivided into two main categories: natural regulatory T cells (nTreg) and induced regulatory T cells (iTreg). The nTreg cells are able to suppress autoimmune diseases and immune responses, and they induce immunological tolerance [49]. The reduction of Treg cells is associated with IBD pathogenesis [78, 79]. Effector T cells may be suppressed through cytokines produced by T regulatory (Treg) cells, which are extremely important for maintaining of the intestinal mucosa homeostasis. They are enrolled in the suppression of the immune responses against an exacerbated number of bacteria. This occurs due to the production of anti-inflammatory cytokines such as IL-10 and TGF-β [80] (Figure 1).

In an experimental study, naive T cells without CD4+ and CD25+ Treg cells were injected into mice with T cell defection. High response to intestinal symbiotic bacteria was verified, which led to the development of an autoimmune colitis [81]. However, when T cells with CD4+ and CD25+ Treg cells were injected into mice models that presented IBD pathological injuries, these cells were recruited to the intestinal lymphatic tissues and to *lamina propria*. They then migrated to the spleen to exert an immune regulation [82].

Tregs perform a huge anti-inflammatory action, as was verified in an experimental study of UC. However, these cells were lacking in the peripheral blood of patients with the active disease, when compared to those who were in the inactive phase or in the control group [83–85]. For Tregs to be functional, a signal made by TGF-β is needed. However, this signal is weakened in IBD due to the upregulation of an inhibitory molecule called Smad7. Fantini et al. [86] observed that the *lamina propria* effector T cells of IBD patients do not respond to Treg signaling. This finding was reversed by the presence of an antisense oligonucleotide anti-Smad7. Therefore, a possible inhibition of Treg cells can contribute to the development of IBD [9].

Reductions of Treg cells were found in peripheral blood and colonic mucosa in IBD patients, suggesting that lower expression of Treg cells is associated with IBD pathogenesis [78, 79].

7. T Helper 17 Cells

For the differentiation and proliferation and Th17 cells, IL-23 act on the IL-23 receptor on the surface of Th cells and activate cytoplasmic signal transduction and transcriptional activation factor 3 (STAT-3). This activation occurs in the presence of TGF-β, IL-6, or IL-21 [49]. The Th17 cells are activated when several cytokines such as IL-17, IL-21, and IL-22 are released. Some clinical studies have found high levels of Th17 and IL-17 in mucosa of IBD patients compared to healthy controls. Th17 cells are mainly distributed in the *lamina propria* of the UC intestinal mucosa and in the submucosa and muscle layer of the mucosa of CD patients [87]. IL-17 is directly associated with the release of proinflammatory factors and also responsible for the induction of immune cell transfer to peripheral tissues. After this process, IL-17 binds to the surface receptors and finally activates NF-kB, releasing proinflammatory factors [49]. It has been observed in studies that showed high IL-17 serum expression in the IBD patients [88].

Th17/Treg cells remain in balance under normal conditions; however, this balance can be disrupted due excessive increases of Th17 cells and decrease of Tregs, leading to damage to the intestinal mucosa [49]. T cells differentiate into Th17 in the presence of IL-6 and low TGF-β concentrations, thereby inhibiting proliferation of Treg cells. On the other hand, high concentrations of TGF-β inhibit Th17 production and increase Treg production [89]. Th17 is increased in the peripheral blood of IBD patients, while Treg cells are decreased, suggesting that the Th17/Treg proportion plays an important role in the development and maintenance of inflammation [49].

8. Intestinal Fibrosis and the Inflammatory Process

Intestinal fibrosis is commonly characterized as an excessive deposition of extracellular matrix (ECM), resulting from chronic inflammation and impaired intestinal wound healing [90]. Inflammation process is necessary for the development of intestinal fibrosis [91]. However, in vivo and in vitro studies suggest that fibrogenic mechanisms can be distinct from the inflammation process. Particularly, in IBD, it is difficult to distinguish the inflammatory response from the fibrotic process, because the cells responsible for each response are intimately associated in the mucosa microenvironment [90].

The main mechanism responsible for the formation of intestinal fibrosis is the growth and increase of the fibroblast population [90]. In IBD, isolated fibroblasts show a faster proliferation rate compared to a non-IBD normal mucosa [92, 93]. In support of this fact, intestinal fibroblasts can increase their growth rate in vitro conditions similar to the inflamed gut [90]. These conditions can activate molecules, such as platelet-derived growth factor (PDGF), insulin like growth factor I (IGF-I), epithelial growth factor (EGF), basic fibroblast growth factor (bFGF), and connective tissue growth factor (CTGF). They also induce the production of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α [90, 92, 94, 95]. After the fibroblasts are recruited, they must be retained at the inflammatory site. This action is mediated by proinflammatory cytokines, such as TNF-α and IFN-γ, and both of them can lead to the fibroblasts' migration in vitro [96]. How much this reduction of the migratory capacity in vivo contributes to the development of fibrosis in IBD is unclear [90]. Therefore, the fibroblasts may also contribute to the intestinal inflammatory conditions in IBD, mainly in CD patients, who are prone to develop fibrostenosis.

9. Conclusion

The immunological aspects of IBD, specifically CD and UC, involve impaired innate and adaptive responses which may be associated with genetic susceptibility, environmental factors, and intestinal microbiota. Th17 cells play an important role in the development and in the maintenance of the disease. Besides, defective anti-inflammatory mechanisms, such as the decrease of Treg cells, are also involved in maintaining the ailment. Moreover, the understanding of the exclusive role of immune cells in all of this process has changed in face of new discoveries, since IECs are also relevant cells in IBD.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

The Role of Genetic and Immune Factors for the Pathogenesis of Primary Sclerosing Cholangitis in Childhood

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Primary sclerosing cholangitis (PSC) is a rare cholestatic liver disease characterized by chronic inflammation of the biliary tree resulting in liver fibrosis. PSC is more common in male less than 40 years of age. The diagnosis of PSC is based on clinical, laboratory, image, and histological findings. A biochemical profile of mild to severe chronic cholestasis can be observed. Endoscopic retrograde cholangiography is the golden standard method for diagnosis, but magnetic resonance cholangiography is currently also considered a first-line method of investigation. Differences in clinical and laboratory findings were observed in young patients, including higher incidence of overlap syndromes, mostly with autoimmune hepatitis, higher serum levels of aminotransferases and gamma-glutamyl transferase, and lower incidence of serious complications as cholangiocarcinoma. In spite of the detection of several HLA variants as associated factors in large multicenter cohorts of adult patients, the exact role and pathways of these susceptibility genes remain to be determined in pediatric population. In addition, the literature supports a role for an altered immune response to pathogens in the pathogenesis of PSC. This phenomenon contributes to abnormal immune system activation and perpetuation of the inflammatory process. In this article, we review the role of immune and genetic factors in the pathogenesis of PSC in pediatric patients.

1. Introduction

Autoimmune liver diseases are infrequent in children and adolescents, but, when diagnosed, prompt treatment has utmost importance. Among these conditions, primary sclerosing cholangitis (PSC) is a rare cholestatic liver disease characterized by chronic inflammation of the biliary tree resulting in liver fibrosis. An important factor for the occurrence of cirrhosis is the toxic effect of bile stasis [1, 2]. In contrast with the female predominance of many autoimmune diseases, approximately 2/3 of the PSC patients are males aged less than 40 years [3].

PSC has a wide-ranging clinical presentation, from asymptomatic patients to chronic liver disease [4, 5]. As a result, the diagnosis can be a big challenge, especially in young patients. Cholestatic bile diseases are usually associated with other autoimmune conditions, including ulcerative colitis (UC) and autoimmune hepatitis, referred to as the overlap syndromes [6, 7]. The named AIH-PSC syndrome is more frequent in children and adolescents than in adult patients; the disease evolves with typical features of PSC in cholagiography or histology, associated with laboratory and histological characteristics of AIH. In AIH-PSC, the reaction to treatment is also different, since patients have better response to immunosuppression than the isolated form of PSC [7].

The diagnosis of PSC is based on clinical, laboratory, and image findings. A biochemical profile of mild to

severe chronic cholestasis can be observed [8]. Endoscopic retrograde cholangiography is the golden standard method to detect PSC. The exam shows multifocal areas of strictures of intra- and/or extrahepatic bile ducts, with intervening segments of normal or dilated ducts [8, 9]. In addition, magnetic resonance cholangiography (MRC) is also considered a first-line image method for PSC diagnosis, being noninvasive and reliable [8, 10, 11].

Multiple autoantibodies can be useful in detecting PSC, but none of them solely allows the diagnosis [12]. Serum atypical perinuclear antineutrophil cytoplasmic antibody (p-ANCA) is the most common autoantibody detected in PSC, but with a weak association [13–15]. The second commonest autoantibodies are anti-*Saccharomyces cerevisiae* antibodies (ASCA), which also exhibit high frequency even in the absence of advanced disease or inflammatory bowel disease (IBD) [16]. Recently, Jendrek et al. [17] also found an association between antibodies against glycoprotein 2 (anti-GP2) and large bile duct diseases with considerable rates. In PSC, anti-GP2 IgA was consistently identified in patients with poor survival during follow-up, being associated with cholangiocarcinoma [17]. Other autoantibodies including antinuclear antibody (ANA) and liver kidney microsomal type 1 antibody (anti-LKM1) can be positive in PSC patients, but with low specificity for the diagnosis of the disease [18].

PSC has no effective medical treatment and, in many cases, the disease will lead to cirrhosis and end-stage liver disease with need of liver transplantation [1, 5, 19, 20]. Ursodeoxycholic acid (UDCA) has been administered as a palliative measure, without interfering with clinical outcome [21, 22]. Since clinical tools have been insufficient to characterize and to predict the outcome, the aim of this review is to summarize evidence from literature about the potential role of immune and genetic factors in the pathogenesis of PSC in pediatric patients.

2. Genetic Factors

The pathogenesis of PSC is still not fully understood, but a complex interaction between genetic, immunological, and environmental factors with breakdown of self-tolerance has been reported [14, 23]. Studies have shown a strong genetic predisposition in PSC, with first-degree relatives exhibiting 9- to 39-fold increased risk to develop the disease [24]. Genome studies showed that this genetic tendency is mainly associated with human leukocyte antigen (HLA) complex II (MHC II) chromosome 6p21 [25–29]. Some haplotypes are considered as main susceptibility factors. HLA-B and HLA-DRB1 alleles are the most important ones. Among them, the most frequent are HLA-A1-B8-DRB1∗0301-DQB1∗0201, HLADRB1∗1301-DCB1∗0603, and HLA-DRB1∗1501-DQB1∗0602 [25–27, 30, 31]. As protective haplotypes, HLA-DRB1∗04-DQB1∗0302 and HLA-DRB1∗0701-DQB1∗0303 have been reported [25, 26, 31]. In spite of the detection of several HLA variants as associated factors in large multicenter cohorts of adult patients, the exact role and pathways of these susceptibility genes remain to be determined in pediatric population [25–29].

Table 1 is a compilation of relevant studies that have investigated the influence of MHC II antigens in PSC. The number of children evaluated in these studies is also shown.

Other studies have proposed the existence of MHC class I region genes and non-HLA risk loci for PSC, supporting that other genetic factors take part in the pathogenesis of the disease. Wiencke et al. [43] evaluated the extended HLA class I region genes as contributing factors to modulate immune response or to confer susceptibility in PSC patients. The authors found a significant association with alleles MIB∗349, D6S265∗122, D6S464∗209, and D6S2225∗147, all being secondary to DR3 (HLA-DRB1∗03) associations [43]. D6S265∗122 was also associated with DR6 [40]. The study of Karlsen et al. [44] showed that killer immunoglobulin-like receptors (KIRs) and HLA class I ligands can be also associated with PSC.

Norris et al. [45] described an association between the *MICA∗008* allele and PSC. *MICA∗008* is one of the components of a group of polymorphic genes on chromosome 6. *MICA∗008* is a stress and heat shock inducible molecule that activates inflammatory response as a ligand for $\gamma\delta$ T cells, natural killer (NK) ($CD56^+$) cells, and cells expressing the NKG2D receptor [42].

Non-HLA findings include modifications in genes related to autoimmunity (IL2/IL2RA), bile acid toxicity (GPBAR1), and mechanisms related to concomitant IBD (IL2/IL21, ILR2A, CARD9, MST1, Fut2, and SIK2) [27, 46]. Karlsen et al. [25] also investigated genome-wide association in PSC. These authors detected a susceptibility variant of importance for inflammatory conditions at chromosome *13q31*, with GPC5/GPC6 as a candidate to this association [25]. Despite its involvement in inflammatory pathways, the precise function of GPC6 in the liver and bile ducts remains unknown and more studies are needed to clarify the role of this molecule.

As shown, most of these studies evaluated only adults, creating a gap in relation to the findings of pediatric patients. Indeed, PSC in children seems to be different from the disease in adults [44, 45]. In this regard, differences in clinical and laboratory findings observed in young patients include higher incidence of overlap syndrome, higher serum levels of aminotransferases and gamma-glutamyl transferase, and lower incidence of serious complications as cholangiocarcinoma [44]. Thus, the results obtained for adult patients may not be always valid for children. Further studies are obviously needed to evaluate the role of immune and genetic factors in pediatric age group. Studies should analyze pediatric patients as a subgroup by separating patients who had disease onset during childhood. This strategy of analysis probably enables more reliable evaluation of the role of immune and genetic factors in pediatric PSC.

3. Immune Factors

The literature supports a role for an altered immune response to pathogens in the pathogenesis of PSC [14, 47]. This phenomenon contributes to abnormal immune system activation and perpetuation of an inflammatory process [14, 47].

Table 1: Publications on major histocompatibility class II human leukocyte antigens and their association with primary sclerosing cholangitis patients.

Reference	Total number of patients/controls (number of children and adolescents)	What was evaluated	Conclusions
Farrant et al. [32], 1992	71/68 (0)	HLADRB, DQA, and DQB	HLADRB3∗0101 was the most associated allele, with reduced survival of patients with it. DRB5∗0101 was another susceptibility allele and DRB4∗0101 demonstrated a likely protective function.
Amar et al. [33], 1992	15/no control (0)	HLADRB3	No apparent association of the alleles of the DRB3 locus in the Israeli population.
Olerup et al. [34], 1995	75/250 (not cited)	HLADR and DQ	Association with DRB1∗1301, DQAI∗0103, and DQBI∗0603 haplotype was confirmed, whereas DRB1∗04 was only slightly underrepresented. No difference was observed in age, presentation, liver function, histological stage, or survival between patients with different positive alleles.
Leidenius et al. [35], 1995	24/106 (not cited)	HLA-A, B, C and DR	HLA-B8 and DR3 (DRB1∗03) were associated with primary sclerosing cholangitis.
Wilschanski et al. [36], 1995	27/no control (all children)	HLA-B and HLADR	An increased incidence of HLA B8 and DR2 (DRB1∗15) but not DRw52a (DRB3∗0101) was found.
Spurkland et al. [30], 1999	256/764 (not cited)	HLADR and DQ	Increased frequencies of DRB1∗03-DQA1∗0501-DQB1∗02, DRB1∗13-DQA1∗0103-DQB1∗0603, and DRB1∗15-DQA1∗0102-DQB1∗0602 haplotypes were observed. PSC was negatively associated with DRB1∗04-DQB1∗0302 haplotype.
Boberg et al. [37], 2001	265/no control (yes, but the number was not cited)	HLADR and DQ	DRB1∗03-DQA1∗0501-DQB1∗02 (i.e., DR3, DQ2) heterozygous genotype was associated with an increased risk of death or liver transplantation. Presence of a DQ6 encoding haplotype (DQB1∗0603 or DQB1∗0602) in DR3, DQ2 negative individuals was associated with a reduced risk of death or liver transplantation.
Donaldson and Norris [31], 2002	148/134 (0)	HLADR and DQ	Associations with the DRB3∗0101-DRB1∗0301-DQA1∗0501-DQB1∗0201 and DRB1∗1301-DQA1∗0103-DQB1∗0603 haplotypes were confirmed. Protective influence of the DRB1∗04-DQB1∗0302 haplotype was reaffirmed. A previously unreported protective haplotype was found: DRB1∗0701-DQB1∗0303.

Table 1: Continued.

Reference	Total number of patients/controls (number of children and adolescents)	What was evaluated	Conclusions
Bittencourt et al. [38], 2002	63/83 (27)	HLA-B, DRB1, DQB1	No increase in the frequency of HLA-B, DRB3, DRB4, or DRB5 alleles was observed. The frequency of HLA-DRB1∗1301 and HLA-DQB1∗06 was significantly increased in PSC patients.
Neri et al. [39], 2003	64/183 (0)	HLA-DRB1, HLA-DQB1, and HLA-B	Frequencies of DRB1∗01-DQA1∗0101-DQB1∗0102, DRB1∗16-DQA1∗0102-DQB1∗0502, and DRB1∗04-DQA1∗03-DQB1∗0301 haplotypes were more elevated in PSC patients. DRB1∗07-DQA1∗0201-DQB1∗02 haplotype frequency was significantly decreased in patients.
Karlsen et al. [25], 2010	285/298 (yes, but the number was not cited)	HLADR and DQ	The strongest association was detected for HLA-B∗08 and associations with the DRB1 alleles -DRB1∗03, -DRB1∗04, -DRB1∗07, and -DRB1∗1301 also were confirmed.
Hov et al. [40], 2010	78/79 (not cited)	HLADRB1, HLA-C	Positive association of PSC with HLADRB1∗15, -DRB1∗03, -DRB1∗04, and -DRB1∗1301 was confirmed. A protective association with HLADRB1∗0701 was found.
Wang et al. [41], 2014	31/42 (all children)	HLADR haplotypes	Frequencies of homozygous HLA DRB1∗0301 (DR3) genes and haplotype A1-B8-DR3 were higher in patients. Frequencies of disease-protective genes DR4 and/or DR15 were lower in the patients.
Næss et al. [42], 2014	365/368 (yes, but the number was not cited)		HLADRB1∗1301-DQB1∗0603 and DRB1∗1501 haplotypes conferred risk for PSC. HLADRB1∗04-DQB1∗03, DRB1∗0701-DQ∗0303, and DR13:XX (all non-13:01 alleles)-DQB1∗06 demonstrated a protective effect.

HLA: human leukocyte antigen; MHC: major histocompatibility complex; PSC: primary sclerosing cholangitis.

Cholangiocytes, after antigenic stimulus, release proinflammatory mediators that stimulate immune cells. The interaction between toll-like receptors (TLRs) and pattern-associated molecular patterns (PAMPs) promotes a persistent inflammatory environment for cholangiocytes [48, 49]. TLR activation can increase the expression of interleukin-6 (IL-6) and of cluster for differentiation 44 (CD44) that acts as an adhesion molecule for lymphocytes [48]. Along with this process, tumor necrosis factor (TNF), IL-6, and IL-8, released by cholangiocytes and immune cells, trigger the recruitment and activation of T lymphocytes, macrophages, neutrophils, NK, and mesenchymal cells [14, 50–53]. In addition, senescence has been recently shown to be an important pathologic process in diverse conditions, since senescence cells can be associated with proinflammatory cytokine and chemokine hypersecretion, referred to as a senescence associated secretory phenotype (SASP) [54]. In this regard, Tabibian et al. [55] showed that PSC cholangiocytes present higher expression of SASP components, including IL-6, IL-8, CCL2, and PAI-1. These cells also had increased expression of N-Ras, a well-known inducer of senescence. These findings support the role of senescence in PSC.

An increased number of activated lymphocytes and of NK cells in peripheral blood of PSC patients were also detected [53, 56]. However, Bo et al. [57] showed that T lymphocytes function is impaired in PSC patients when compared with health controls. In regard to NK cells, it was reported that these cells are activated by lipid antigens exposed by the MHC class I-like molecule, CD1d [58]. When activated, NK cells can play either a protective or detrimental role in autoimmune diseases [58]. In a recent study, Schrumpf et al. [59] reported that human cholangiocytes can express CD1d and that CD1d is downregulated in biliary epithelium of patients with PSC. These authors also showed that cholangiocytes unmask lipid antigens to NL cells, suggesting that this mechanism could play a role in the autoreactivity of NK cells in PSC [59].

There are some hypotheses about how cytokines and adhesion molecules may contribute to PSC pathogenesis. In this context, lymphocytes express specific receptors, $\alpha 4\beta 7$ and CCR9, with these cells being responsible for interferon-γ (IFN-γ) production, which, in turn, enhances inflammatory stimuli [60, 61]. Additionally, due to the release of cytokines as CCL28, CXCL12, and CXCL16 and the presence of activated lymphocytes, *naive* T cells can be primed to T helper 1-cells [62, 63]. In line with these findings, Martins et al. [64] showed that $\gamma\delta^+$ cells expressing CD45RO and IL-2 contribute to an activated memory mechanism that maintains the inflammatory process. Sebode et al. [65] had also described a reduction of regulatory immune cells, including $FOXP3^+$ cells and T reg cells, in PSC patients. Indeed, an increased production of inflammatory cytokines including IL-17, IL-21, and TNF and of IL-17A-expressing lymphocytes can be found around bile ducts [66]. In this regard, Liu et al. [29] showed novel loci related to cytokines and chemokines in patients with PSC. The authors detected six loci in which the same gene was found by more than one technique, supporting the role of these genes in PSC [29]. At position 11q23, the most strongly associated single nucleotide polymorphism (SNP), rs7937682, is located in an intron of *SIK2* gene, which encodes salt-inducible kinase 2. This protein influences the expression of both IL-10 in macrophages and Nur77, an important transcription factor in leukocytes [29]. Another relevant association was found at 12q13, which is an intronic SNP, rs11168249, within the *HDAC7* gene, that encodes histone deacetylase 7 [29]. This molecule has been implicated in the negative selection of T cells in the thymus, a key process in the development of immune tolerance. More studies are needed to elucidate the clinical value of these genetic findings and whether these genes can also be related to pediatric PSC.

The CCR5 (chemokine receptor 5) is responsible for the recruitment of activated lymphocytes via portal expression of CCR5 ligands [67, 68]. CCR5 also contributes to generation of T helper 1 immune response [68, 69]. Despite controversial findings, some studies report that CCR5-Delta32 deletion is associated with significant reduction in cell surface expression of this chemokine receptor, thus compromising lymphocytes activation [67–69]. While Eri et al. [68] showed that CCR5-Delta32 allele frequency was significantly higher in PSC compared to controls, Melum et al. [69] did not find any statistically significant difference in the occurrence of this mutation in patients and controls.

Some studies showed that mucosal addressin cell adhesion molecule (MAdCAM-1) plays an important role in T-lymphocyte recruitment to liver tissue derived from the gut by the connection with $\beta 2$-integrin ligand [61, 67, 70]. This mechanism explains the hepatic recruitment of mucosal lymphocytes in inflammatory liver diseases. For patients with IBD and associated PSC, this pathway has been considered as a potential therapeutic target [71]. There are studies evaluating antiadhesion molecule therapies, but yet without convincing results [71].

Liaskou et al. [72] showed that when compared with $CD28^+$ T cells, activated $CD28^-$ T cells produce high levels of IFNγ and TNF, leading to upregulation of intercellular cell adhesion molecule-1, HLA-DR, and CD40, which are important ligands for immune activated T cells. The authors also described significantly greater proportion of $CD4^+CD28^-$ and $CD8^+CD28^-$ cells that infiltrate in liver tissue of patients with PSC, leading to a proinflammatory environment rich in TNF [72].

4. Concluding Remarks

In conclusion, HLA class I and class II are shown to be the main risk factors for PSC in the MHC, but it is time to consolidate available information and to translate research findings into applicable knowledge for clinical practice. Studies with children are infrequent, and the findings obtained in adults should not be extrapolated for this age group. Changes in immune response to pathogens, activation of T lymphocytes, and release of inflammatory and adhesion molecules also contribute to the pathogenesis of PSC. More studies are clearly needed to unveil the influence of genetic and immune factors in the pathogenesis of PSC in pediatric patients and how these markers can be used as diagnostic tools and/or therapeutic targets.

Competing Interests

There is no conflict of interests associated with any of the senior author or other coauthors.

Authors' Contributions

D. M. Miranda, E. D. Fagundes, A. R. Ferreira, and A. C. Simões e Silva designed the research; P. M. Ferri, S. L. C. Silva, and D. J. Q. Aquino performed the research; D. M. Miranda, P.M. Ferri, A. R. Ferreira, and A. C. Simoes e Silva wrote the paper; all authors reviewed the final version.

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