Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 05, pp.6768-6771, May, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INVESTIGATING THE ROLE OF IL-31 AND IL-33 IN INFLAMMATORY BOWEL DISEASE

[1]Maha F. Nazzal, Hazima M. K., [1]Al-Abassi Ali, H. and *[2]Ad'hiah

[1]Department of Biology, College of Education Ibn Al-Hitham, University of Baghdad, Baghdad, Iraq
[2]Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

ARTICLE INFO

Article History:
Received 20th February, 2014
Received in revised form
15th March, 2014
Accepted 29th April, 2014
Published online 31st May, 2014

Key words:

Inflammatory, Bowel Disease,
IL-31, IL-33.

ABSTRACT

Serum level of IL-31 and IL-33 was assessed in sera of 79 inflammatory bowel disease (IBD) Iraqi patients; 54 ulcerative colitis (UC) and 25 crohn's disease (CD), as well as 10 apparently healthy controls. The patients attended the Gastrointestinal Teaching Hospital in Baghdad for diagnosis and treatment during the period March-August 2012. Serum level of IL-31 was approximated in UC and CD patients and controls (1.69 ± 0.09, 1.71 ± 0.31 and 1.56 ± 0.24 pg/ml, respectively). In contrast, IL-33 was significantly increased ($P \leq 0.001$) in UC and CD patients (3.29 ± 0.44 and 6.41 ± 0.18 pg/ml, respectively) compared to controls (0.74 ± 0.28 pg/ml). These results suggest a role of IL-33 in the pathogenesis of both clinical groups of IBD, especially CD.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of intestinal inflammatory diseases that can be subdivided into ulcerative colitis (UC) and Crohn's disease (CD) on the basis of typical clinical manifestations. The former is a transmural chronic inflammation potentially affects any gastrointestinal tract from mouth to anus, while the latter is a non-transmural disease affecting the colon with a caudo-cranial extension without patchiness or skip lesions (Podolsky, 2002). In CD, all layers of the gut may be involved and normal healthy gut may be found between sections of diseased bowel. In contrast, UC causes inflammation and ulcers in top layer lining the large intestine (Danese and Fiocchi, 2006). Studies in experimental animal models indicated that IBD-related tissue damage results from a dynamic interplay between immune and non-immune cells, in which cytokines are crucial mediators in this interplay. Cytokines are low molecular weight proteins produced mainly by immune cells that facilitate communication between cells, stimulate proliferation of antigen-specific effector cells, and mediate local and systemic inflammation (Strober and Fuss, 2011), and play an important role in IBD because they are key signaling molecules of intestinal immune system (Sanchez-Muñoz *et al*., 2008).

Cytokines may be involved in pathogenesis of IBD (Kaser *et al*., 2010), and it is also evident that these mediators play an important role in local, as well as, in the systemic acute-phase response of the inflammatory process in IBD (Műzes *et al*., 2012). The present study focused on two of these cytokines, which were IL-31 and IL-33. IL-31 was described in 2004 as a 24 kDa glycoprotein of short-chain 4-helix bundle cytokine consisting of 141 amino acids that is expressed by activated CD4+ T cells; preferentially by T cells that are skewed toward a Th2 phenotype (Dillon *et al*., 2004). Recently, it has been demonstrated that human mast cells are also a source of IL-31 (Niyonsaba *et al*., 2010). Moreover, it has been demonstrated that monocytes, macrophages, immature and especially mature monocyte-derived DCs can produce IL-31 in response to oxidative stress (Cornelissen*et al*., 2011). The available data suggests that IL-31 is important for both innate and adaptive immunity in tissues that are in close contact with the environment, i.e. skin, airways and lung, and the lining of intestine, and accordingly, it has been suggested that enhanced expression of IL-31 might be associated with a number of diseases, including IBD (Cornelissen *et al*., 2012).

In 2003, a novel 30 kDa protein, localized in the nuclei of endothelial cells, was identified, and this protein was hypothesized to possess nuclear factor function, critical for the induction of a lymphatic endothelium phenotype, and was therefore coined "Nuclear Factor-High Endothelial Venules" (NF-HEV) (Baekkevold *et al*., 2003). Two years later, NF-HEV was identified as a novel member of the IL-1 cytokine family, shown to be the ligand for the former receptor, ST2, and renamed IL-1F11 or IL-33 (Schmitz *et al*., 2005). Later studies characterized it as a potent inducer of the Th2-

Corresponding author: Ad'hiah
Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq.

associated cytokines IL-4, IL-5 and IL-13 released from polarized Th2 cells, mast cells and basophils (Smithgall *et al*., 2008). Functionally, IL-33 has been considered as a cytokine with dual function, acting as a pro-inflammatory cytokine and as an intracellular nuclear factor with transcriptional regulatory properties (Carriere *et al*., 2007). It is expressed in various types of cells, including epithelial cells, endothelial cells, fibroblasts and smooth muscle cells (Moussion *et al*., 2008). IL-33 has also been reported to be localized in barrier epithelia within organs/tissues in direct contact with the external environment, including the skin, airway, and gut epithelia, suggesting a possible role of this cytokine in early immune responses against invasive pathogens, and may induce an inflammatory response in mucosal epithelium (Saenz *et al*., 2008). As such, IL-33 appeared to be a promising candidate to be studied in the setting of human IBD. Accordingly, the present study determined the serum level of IL-31 and IL-33 in a sample of Iraqi IBD patients.

MATERIALS AND METHODS

Patients

The study was approved by the Medical Ethics Committee of the Ministry of Health in Iraq, in which 79 Iraqi patients with IBD were investigated. The patients attended the Gastrointestinal Tract Unit at Al-kadhamyiah Teaching Hospital in Baghdad for diagnosis and treatment during the period March - August 2012. The disease was clinically diagnosed by the consultant medical staff at the hospital, which was based on a clinical evaluation using colonoscopy and a histopathological examination of a biopsy. According to the diagnosis of consultants, the patients were clinically distributed into two clinical groups: UC, which included 54 patients (34 males and 20 females), and their age mean ± S.E. was 38.9 ± 1.7 years; and CD, which included 25 cases (15 males and 10 females), and their age mean ± S.E. was 37.8 ± 2.2 years. In addition to patients, 10 apparently healthy controls of blood donors (5 males and 5 females) matched patients for age (34.4 ± 3.7 years) and ethnicity (Iraqi Arabs) were also enrolled in the study.

Assessment of IL-31 and IL-33 serum levels

Five milliliters of venous blood was collected from each participating subject. The blood sample was immediately transferred to a plain tube and left to clot at room temperature (20-25°C) for 15 minutes. Then, it was centrifuged at 1000 rpm for 10 minutes to separate serum, which was distributed into aliquots and stored frozen at -20°C until assayed for IL-31 and IL-33 level in sera. The assessment was carried out by using two ELISA kits (Peprotech, UK). They were designed for a quantitative measurement of human IL-31 and IL-33 in serum, in which an anti-human IL-31 or IL-33 coating antibody (Capture Antibody) was adsorbed onto wells of 96-well plate. Human cytokine present in the sample or standard binds to antibodies that were adsorbed to the wells. A biotinylated anti-human IL-31 or IL-33 antibody was then added and binds to human cytokine captured by the first antibody (Detection Antibody). Following incubation, unbound biotinylated anti-human cytokine antibody was removed during a wash step, and avidin horseradish peroxidase (HRP) conjugate was then added and binds to the biotinylated anti-human cytokine antibody. Following incubation, unbound avidin-HRP conjugate was removed during a wash step, and a substrate solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of human cytokine present in the sample or standard. The color development was monitored with ELISA plate reader and absorbance was measured at a wavelength of 405 nm. A standard curve was plotted from standard IL-31 and IL-33, and their level was determined from a curve fitting equation.

Statistical analyses

Serum level of IL-31 and IL-33 was analyzed using the SPSS (Statistical Package for Social Sciences) version 13. Their data were given as mean ± standard error (S.E.), and differences between means were assessed by ANOVA (Analysis of Variance), followed by LSD (Least Significant Difference).

RESULTS

Serum level of IL-31 was approximated in UC and CD patients and controls (1.69 ± 0.09, 1.71 ± 0.31 and 1.56 ± 0.24 pg/ml, respectively). In contrast, IL-33 was significantly increased ($P \leq 0.001$) in UC and CD patients (3.29 ± 0.44 and 6.41 ± 0.18 pg/ml, respectively) compared to controls (0.74 ± 0.28 pg/ml) (Tables 1 and 2). IL-33 also showed a significant increased level in CD patients compared UC patients (Table 3).

Table 1. Mean level of IL-31and IL-33 in sera of ulcerative colitis patients and controls

Cytokine	Serum Level (Mean ± S.E.; pg/ml)		P ≤	95% Confidence Interval
	Controls (No. = 10)	Ulcerative Colitis (No. = 54)		
IL-31	1.56 ± 0.24	1.69 ± 0.09	N.S.	-0.34 - 0.6
IL-33	0.74 ± 0.28	3.29 ± 0.44	0.001	0.48 - 4.628

N.S.: Not significant (P > 0.05)

Table 2. Mean level of IL-31 and IL-33 in sera of Crohn's disease patients and controls

Cytokine	Serum Level (Mean ± S.E.; pg/ml)		P ≤	95% Confidence Interval
	Controls (No. = 10)	Crohn's Disease (No. = 25)		
IL-31	1.56 ± 0.24	1.71 ± 0.13	N.S.	-0.37 - 0.67
IL-33	0.74 ± 0.28	6.41 ± 0.18	0.001	4.99 - 6.359

N.S.: Not significant (P > 0.05)

Table 3. Mean level of IL-31 and IL-33 in sera of ulcerative colitis and Crohn's disease patients

Cytokine	Serum Level (Mean ± S.E.; pg/ml)		P ≤	95% Confidence Interval
	Ulcerative Colitis (No. = 54)	Crohn's Disease (No. = 25)		
IL-31	1.69 ± 0.09	1.71 ± 0.13	N.S.	-0.30 - 0.34
IL-33	3.29 ± 0.44	6.41 ± 0.18	0.001	1.81 - 4.44

N.S.: Not significant (P > 0.05)

DISCUSSION

The present results demonstrated that IL-31 might not have an effect on the pathogenesis of IBD, while IL-33 may impact the pathogenesis of both clinical groups of IBD (UC and CD), in which a significant increased serum level was observed, but the increase was more pronounced in CD (8.5 fold) than in UC patients (2.5 fold) compared to controls. In agreement with these findings, Beltrán *et al*. (2010) found increased IL-33 levels in the serum of patients with IBD, but no correlation with disease activity was observed. In contrast, Ajduković *et al*. (2010) found no difference in IL-33 serum levels between 18 UC patients and healthy controls; suggesting that the role of IL-33 in UC might be posttranscriptional since they could not find any increase in cytokine levels in affected subjects. However, Pastorelli *et al*. (2010) clearly demonstrated that IL-33 serum levels were higher in UC and CD patients compared to controls; an observation that support the present study results. In a further study, only the cleaved form of IL-33 was detectable in humans, and accordingly the authors suggested that the cleaved form of IL-33 could serve as a circulating biomarker, particularly in the UC patients (Pastorelli *et al*. 2011).

In addition, it has also been showed that anti-TNF therapy could modulate IL-33 serum levels in IBD patients, and to evaluate the impact of anti-TNF therapy on IL-33 levels in sera, samples were collected prior to and after infliximab infusions. In the experiment, an acute effect of anti-TNF was detected with a subsequent decline in systemic IL-33 levels. Importantly, circulating IL-33 remained at reduced levels during maintenance therapy, showing that such treatment has long-lasting effects on IL-33 serum levels (Pastorelli *et al*., 2013). However, the clinical and prognostic consequence of the aforementioned effect remains to be established by larger cohort studies (Nunes *et al*., 2014). In intestinal mucosa, Beltrán *et al*. (2010) showed for the first time that patients with UC had higher IL-33 protein levels in intestinal mucosa compared with CD subjects and healthy controls regardless of disease activity. At RNA level, IL-33 mRNA was also upregulated in UC compared with controls using isolated epithelial cells (Seidelin *et al*., 2010), whole biopsy tissue (Kobori *et al*., 2010), and surgical specimens (Sedhom *et al*., 2012). It has been further suggested that IL-33 expression is not only upregulated in IBD mucosa, but it also correlates with the inflammatory status. In this regard, Beltrán *et al*. (2010) observed increased levels of IL-33 protein in biopsy extracts of active UC patients compared with patients in remission. At the same year, Kobori *et al*. (2010) were the first to report an increase in IL-33 expression in mRNA levels in the intestinal mucosa of UC patients with active disease compared with subjects in remission. Seidelin *et al*. (2010) also found increased mRNA levels of IL-33 in active UC compared with patients in remission and controls using RNA from isolated epithelial cells. These results were further confirmed by Sponheim *et al*. (2010), who found elevated levels of IL-33 mRNA in colonic biopsy samples from UC subjects compared with controls and observed that IL-33 values were correlated with clinical activity scores and also with the endoscopic level of inflammation. However, the abnormal expression or dynamic changes of IL-33 represent a primary defect or a secondary phenomenon in the IBD pathogenesis remains to be established, and further studies will be necessary in order to thoroughly investigate the exact role of IL-33 in human IBD and other chronic inflammatory diseases involving the gastrointestinal tract.

REFERENCES

Ajduković, J., Tonkić, A., Salamunić, I., Hozo, I., Šimunić, M., and Bonacin, D. 2010. Interleukins IL-33 and IL-17/IL-17A in patients with ulcerative colitis. *Hepato-Gastroenterology*, 57: 1442-1444.

Baekkevold, E. S. Roussigné, M. Yamanaka, T., Johansen, F.E., Jahnsen, F.L., Amalric, F., Brandtzaeg, P., Erard, M., Haraldsen, G. and Girard, J.P. 2003. Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. *American Journal of Pathology*, 163: 69-79.

Beltrán, C. J., Núñez, L. E., Díaz-Jiménez, D., Farfan, N., Candia, E., Heine, C., López, F., González, M.J., Quera, R. and Hermoso MA.. 2010. Characterization of the novel ST2/IL-33 system in patients with inflammatory bowel disease. *Inflamm. Bowel. Dis*., 16: 1097-1107.

Carriere, V., Roussel, L., Ortega, N., Lacorre, D.A., Americh, L., Aguilar, L., Bouche, G. and Girard, J.P. 2007. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc. Natl. Acad. Sci. U. S. A*., 104: 282-287.

Cornelissen, C., Brans, R., Czaja, K., Skazik, C., Marquardt, Y., Zwadlo-Klarwasser, G., Kim, A., Bickers, D.R., Lüscher-Firzlaff, J., Lüscher, B., Baron, J.M. and Ultraviolet, B. 2011. (UVB) radiation and reactive oxygen species (ROS) modulate IL-31 expression in T-lymphocytes, monocytes and dendritic cells. *Br. J. Dermatol*., 165: 966-975.

Danese, S. and Fiocchi, C. 2006. Etiopathogenesis of inflammatory bowel diseases. *World J. Gastroenterol.*, 12: 4807-4812.

Kaser, A., Zeissig, S. and Blumberg, R.S. 2010. Inflammatory bowel disease. *Annu. Rev. Immunol.*, 28: 573-621.

Kobori, A., Yagi, Y., Imaeda, H., Ban, H., Bamba, S., Tsujikawa, T., Saito, Y., Fujiyama, Y. and Andoh, A. 2010. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J. Gastroenterol.*, 45: 999-1007.

Moussion, C., Ortega, N. and Girard, J.P. 2008. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in *vivo*: a novel 'alarmin'?. *PLoS One*, 3: e3331.

Műzes, G., Molnár, B., Tulassay, Z. and Sipos, F. 2012. Changes of the cytokine profile in inflammatory bowel diseases. *World J. Gastroenterol*., 18: 5848-5861.

Niyonsaba, F., Ushio, H., Hara, M., Yokoi, H., Tominaga, M., Takamori, K., Kajiwara, N., Saito, H., Nagaoka, I., Ogawa, H. and Okumura K. 2010. Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. *J. Immunol*., 184: 3526-3534.

Nunes, T., Etchevers, M.J., Sandi, M.J., Pinó Donnay, S., Grandjean, T., Pellisé, M., Panés, J., Ricart, E., Iovanna, J.L., Dagorn, J.C., Chamaillard, M. and Sans, M. 2014. Pancreatitis-associated protein does not predict disease

relapse in inflammatory bowel disease patients. *PLoS One,* 9: e84957.

Pastorelli L., de Salvo, C. Vecchi, M. and Pizarro, T. T. 2013. The role of IL-33 in gut mucosal inflammation. *Mediators of Inflammation*, 2013: 11.

Pastorelli, L., de Salvo, C., Cominelli, M. A., Vecchi, M., and PizarroT. T. 2011. Novel cytokine signaling pathways in inflammatory bowel disease: insight into the dichotomous functions of IL-33 during chronic intestinal inflammation. Therapeutic Advances in Gastroenterology, 4: 311-323.

Pastorelli, L., Garg, R. R., Hoang, S.B. Spina, L., Mattioli, B., Scarpa, M., Fiocchi, C., Vecchi, M., Pizarro, T.T. 2010. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. *Proc. Natl. Acad. Sci. U.S.A.*, 07: 8017-8022.

Podolsky, D.K. 2002. Inflammatory bowel disease. *N. Engl. J. Med*. 347: 417-29.

Saenz, S.A., Taylor, B.C. and Artis. D. 2008. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol. Rev.*, 226: 172-190.

Sanchez-Munoz, F., Dominguez-Lopez, A. and Yamamoto-Furusho, J.K. 2008. Role of cytokines in inflammatory bowel disease. *World J. Gastroenterol*., 14: 4280-4288.

Schmitz, J. Owyang, A. Oldham, E., Song, Y., Murphy, E., McClanahan, T.K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D.M., Bazan, J.F. and Kastelein, R.A. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*., 23: 479-490.

Seidelin, J. B., Bjerrum, J. T., Coskun, M., Widjaya, B., Vainer, B. and Nielsen, O. H. 2010. IL-33 is upregulated in colonocytes of ulcerative colitis. *Immunology Letters*., 128: 80-85.

Smithgall, M.D., Comeau, M.R., Yoon, B.R., Kaufman, D., Armitage, R. and Smith, D.E. 2008. IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells. *Int. Immunol*., 20: 1019-1030.

Sponheim, J., Pollheimer, J., Olsen, T., Balogh, J., Hammarström, C., Loos, T., Kasprzycka, M., Sørensen, D.R., Nilsen, H.R., Küchler, A.M., Vatn, M.H. and Haraldsen, G. 2010. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am. J. Pathol*., 177: 2804-2815.

Strober, W. and Fuss, I.J. 2011. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology,* 140: 1756-1767.

****