1130-0108/2003/95/Nº Extraordinario/65-67
Revista Española de Enfermedades Digestivas

Rev Esp Enferm Dig (Madrid)
Vol. 95. N.° Extraordinario, pp. 65-67, 2003

Change and progress in inflammatory bowel disease

A. S. Peña

Department of Gastroenterology and Head of the Laboratory of Immunogenetics. VU University Medical Centre. Amsterdam, The Netherlands

INTRODUCTION

Ulcerative colitis and Crohn's disease are complex diseases of unknown aetiology. However, but there is firm evidence that inflammatory bowel diseases are multifactorial and polygenic in nature. Several genes are involved in the susceptibility. To suffer from these diseases, certain genes in different chromosomes are necessary, but they are not sufficient by themselves, to cause these diseases. The genome-wide approach studies lead to a gene coding for NOD2, the now called CARD15 *gene, the first gene involved in the susceptibility of Crohn's disease.*

The genome-wide studies in IBD have already confirmed the polygenic and multifactorial nature of the diseases (1-3). Originally it was thought that CARD15/NOD2 was only expressed in monocytes but recent evidence demonstrates its expression in intestinal epithelial cells as well (4, 5). It is now known that mutations in the CARD15 *gene may serve as a key component of innate mucosal responses to luminal bacteria. Failure in this activity may contribute to the development of Crohn's disease (4). Mutations of intestinal genes regulating the innate immunity are influenced by the microbiological ecology of the individual. Therefore, the frequency of the mutations will vary in different populations. For example, a single nucleotide polymorphism (Asp299Gly) in the Toll-like receptor-4* (TLR-4) *gene that affects the responsiveness to lipopolysaccharide in humans is present in about 10% of the western population (6), but this mutation could not be detected in 275 subjects of the Japanese population (7) neither in a similar number of subjects in China (Xia Bing, Wuhan, personal communication). Similarly, concerning the* NOD2/CARD15 *mutations a novel haplotype conferring risk for Crohn's disease, has been found only in Ashkenazi Jews (8) and the frequent mutations found in the western population do not exist in the Japanese (9, 10), Korean (11), Chinese (12), or in the Cretan populations of patients with Crohn's disease (13). Interesting data is coming from Spain in relation to the frequency of these mutations, while the frequency of the patients studied in Madrid who are mainly from Castilla is similar to that observed in other Caucasian populations (Juan Luis Mendoza et al. Scand J Gastroenterol in press) a different distribution has been found in the homogeneous population of Galicia (Conchita Núñez et al. Amer J Gastroenterol in press). The allele frequencies of* CARD15 *variants were lower in this population than in most of the European populations studied so far. G908R and 1007fs were significantly associated with overall susceptibility to Crohn's disease; these frequencies appear more similar to that reported in Scotland (Satsangi, personnal communication) and Finland (14).*

The findings on the genetic susceptibility of Crohn's disease have shifted the focus of research in this disease from the acquired immunity to the innate immunity (15-20). Advances on the innate immunity and its relationship with the acquired immunity are providing a good insight in the mechanisms that control the inflammation in the gut. The identification and functional characterization, including the discovery of mutants which completely abolish nuclear factor κB (NFκB) signal transduction, like the extracellular Toll-like receptors (TLRs) and the intracellular NOD/CARDs receptors, are providing a new insight in the relationship of the bacteria and the host and in the relationship of the bacteria and the development of disease (20, 21). The Toll-related proteins, of which 10 have been identified, are highly conserved through evolution and are expressed in both enterocyte and immune cells. They recognize specific microbial components through leucine rich region domains (LRRs), such as surface determinants, lipopolysaccharide (LPS) of Gram-negative bacteria (TLR2 and TLR4) and unmethylated CpG DNA sequences (TLR9). Their activation induces the production of T-helper 1 (TH1) cytokines through a process dependent on NFkB; (22-24). LPS and the Lipid A fraction

of LPS is recognized by TLR4. The D299G mutation, affecting the LRR domain of TLR4, is associated with a blunted response to inhalated LPS with a dominant genetic effect. An increased expression of this receptor in the epithelial cells of patients with IBD is related to intestinal flora (15). Other products of intestinal bacterial flora such as peptidoglycan of Gram-positive bacteria are able to stimulate specific receptors, the family of NOD proteins of the cytoplasm of antigen presenting cells, which are also characterized by the presence LRRs. Both receptors are able to stimulate the NFκB, a key immunoregulatory molecule that regulates the inflammatory response. The presence of caspase activating regions in NOD proteins suggests their importance in apoptosis (18, 25, 26).

From the clinical significance point of view and from the basic science, the story is still incomplete. At present it can be concluded that the findings of the research in the genetics and immunology (immunogenetics) are leading to a deeper understanding of the biology and pathophysiology of inflammatory bowel disease.

As Gordon Duff (27) has stated: "the ability to screen for genetic susceptibility, brings with it the possibility of modifying risk by chemoprevention, dietary manipulation, choice of work environment, and lifestyle adjustments".

These ideas with the advances in new technology have contributed to stimulate the clinical investigators interested in the genetics of IBD in this direction. Although, a wise note of caution has been raised by Dr. N. Holtzman: "exaggerating the importance of genetic factors stops people thinking about the need to clean up the environment and tackle socioeconomic inequity" (27) and we need to keep the right balance for the benefit of our patients.

Another major advance has recently occurred that will have a significant impact in IBD research. A wide range of culturing and molecular methods (16S rRNA) are now available for studying bacterial populations in the large intestine. Molecular investigations have allowed analysis of bacterial populations in the colon that were unknown (28-30). FISH in combination with CLSM allows bacterial colonisation of mucosal surfaces and biofilm formation to be visualised. This will be useful for studies in IBD. Culturing studies in combination with chemotaxonomic and molecular methods of analysis indicate that bifidobacterial, enterococcal and peptostreptococcal populations are significantly different in UC (31). Analysis of mucosal bacterial communities using real-time PCR will provide a rapid, sensitive and high throughput method for quantitating bacteria colonising the gut epithelium. The most interesting advances in this field that offer ground for future studies in the years to come is the parallel advances in the NBS-LRR proteins (for nucleotide-binding site and leucine-rich repeat) which are involved in intracellular recognition of microbes and their products. NBS-LRR proteins are characterized by three structural domains: a C-terminal leucine-rich repeat (LRR) domain able to sense a microbial motif. The intermediary nucleotide binding site (NBS) essential for the oligomerization of the molecule that is necessary for the signal transduction induced by different N-terminal effector motifs, such as a caspase-activating and recruitment domain (CARD) (32, 33). These advances will no doubt give new impulse to the studies of probiotics in understanding gut ecology (34) and in the management of IBD (35).

IBD is clinically heterogeneous, with varying natural history and response to treatment (36). Despite much effort to identify genetic, serological markers and clinical measures of risk, methods to accurately predict a patient's clinical course are not yet available. We are, however, at present at an important turning point in the introduction of new molecular advances in the field of genetics and immunology. Advances in experimental IBD offer new tools and approaches to understand the complicated immunological response of the gut and the polygenic response (37). Gene-expression profiling by DNA microarrays produce gene-expression measurements for thousands of genes simultaneously. It is to be expected that in the years to come, genetic information from experimental animal models, DNA and RNA microarrays will lead to a deeper understanding of the molecular pathways that cause chronic inflammation and in turn will help to design drugs with a clearer rationale (38).

REFERENCES

1. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411: 599-603.
2. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411: 603-6.
3. Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, et al. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. Lancet 2001; 357: 1925-8.
4. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 2003; 124: 993-1000.
5. Rosenstiel P, Fantini M, Brautigam K, Kuhbacher T, Waetzig GH, Seegert D, et al. TNF-alpha and IFN-gamma regulate the expression of the NOD2 (CARD15) gene in human intestinal epithelial cells. Gastroenterology 2003; 124: 1001-9.
6. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000; 25: 187-91.
7. Okayama N, Fujimura K, Suehiro Y, Hamanaka Y, Fujiwara M, Matsubara T, et al. Simple genotype analysis of the Asp299Gly polymorphism of the Toll-like receptor-4 gene that is associated with lipopolysaccharide hyporesponsiveness. J Clin Lab Anal 2002; 16: 56-8.
8. Sugimura K, Taylor KD, Lin YC, Hang T, Wang D, Tang YM, et al. A novel NOD2/CARD15 haplotype conferring risk for Crohn disease in Ashkenazi Jews. Am J Hum Genet 2003; 72: 509-18.
9. Inoue N, Tamura K, Kinouchi Y, Fukuda Y, Takahashi S, Ogura Y, et al. Lack of common NOD2 variants in Japanese patients with Crohn's disease. Gastroenterology 2002; 123: 86-91.

10. Yamazaki K, Takazoe M, Tanaka T, Kazumori T, Nakamura Y. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. J Hum Genet 2002; 47: 469-72.
11. Croucher PJ, Mascheretti S, Hampe J, Huse K, Frenzel H, Stoll M, et al. Haplotype structure and association to Crohn's disease of CARD15 mutations in two ethnically divergent populations. Eur J Hum Genet 2003; 11: 6-16.
12. Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, et al. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther 2003; 17: 1465-70.
13. Roussomoustakaki M, Koutroubakis I, Vardas EM, Dimoulios P, Kouroumalis EA, Baritaki S, et al. NOD2 insertion mutation in a Cretan Crohn's disease population. Gastroenterology 2003; 124: 272-273; author reply 273-4.
14. Helio T, Halme L, Lappalainen M, Fodstad H, Paavola-Sakki P, Turunen U, et al. CARD15/NOD2 gene variants are associated with familially occurring and complicated forms of Crohn's disease. Gut 2003; 52: 558-62.
15. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68: 7010-7.
16. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA. Nature 2000; 408: 740-5.
17. Beutler B. Not "molecular patterns" but molecules. Immunity 2003; 19: 155-6.
18. Beutler B. Autoimmunity and apoptosis: the Crohn's connection. Immunity 2001; 15: 5-14.
19. Hecht G, Koutsouris A. Myosin regulation of NKCC1: effects on cAMP-mediated Cl- secretion in intestinal epithelia. Am J Physiol 1999; 277: C441-7.
20. Hecht G. Innate mechanisms of epithelial host defense: spotlight on intestine. Am J Physiol 1999; 277: C351-8.
21. Cho JH. The Nod2 gene in Crohn's disease: implications for future research into the genetics and immunology of Crohn's disease. Inflamm Bowel Dis 2001; 7: 271-5.
22. Giacomini E, Iona E, Ferroni L, Miettinen M, Fattorini L, Orefici G, et al. Infection of human macrophages and dendritic cells with Mycobacterium tuberculosis induces a differential cytokine gene expression that modulates T cell response. J Immunol 2001; 166: 7033-41.
23. Korhonen R, Korpela R, Moilanen E. Signalling mechanisms involved in the induction of inducible nitric oxide synthase by Lactobacillus rhamnosus GG, endotoxin, and lipoteichoic acid. Inflammation 2002; 26: 207-14.
24. Murillo LS, Morre SA, Pena AS. Toll-like receptors and NOD/CARD proteins: Pattern recognition receptors are key elements in the regulation of immune response. Drugs Today (Barc) 2003; 39: 415-38.
25. Inohara N, Nunez G. The NOD: a signaling module that regulates apoptosis and host defense against pathogens. Oncogene 2001; 20: 6473-81.
26. Inohara N, Nunez G. NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol 2003; 3: 371-82.
27. Burn J, Duff G, Holtzman N. Three views of genetics: the enthusiast, the visionary, and the sceptic. Interview by Tessa Richards. BMJ 2001; 322: 1016.
28. Hopkins MJ, MacFarlane GT. Evaluation of 16s rRNA and cellular fatty acid profiles as markers of human intestinal bacterial growth in the chemostat. J Appl Microbiol 2000; 89: 668-77.
29. Hopkins MJ, Sharp R, Macfarlane GT. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. Gut 2001; 48: 198-205.
30. Hopkins MJ, Sharp R, Macfarlane GT. Variation in human intestinal microbiota with age. Dig Liver Dis 2002; 34 (Supl. 2): S12-18.
31. Cummings JH, Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. Curr Issues Intest Microbiol 2003; 4: 9-20.
32. Chamaillard M, Girardin SE, Viala J, Philpott DJ. Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. Cell Microbiol 2003; 5: 581-92.
33. Inohara N, Ogura Y, Nunez G. Nods: a family of cytosolic proteins that regulate the host response to pathogens. Curr Opin Microbiol 2002; 5: 76-80.
34. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003; 361: 512-9.
35. Karimi O, Peña AS. Probiotics: isolated bacteria strain or mixtures of different strains? Two different approaches in the use of probiotics as therapeutics. Drugs of Today 2003; 39: 565-97.
36. Gasche C, Alizadeh BZ, Pena AS. Genotype-phenotype correlations: how many disorders constitute inflammatory bowel disease? Eur J Gastroenterol Hepatol 2003; 15: 599-606.
37. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3: 521-33.
38. Rutgeerts P. Modem therapy for inflammatory bowel disease. Scand J Gastroenterol Suppl. 2003: 30-3.