Epidemiologic studies suggest that NSAIDs increase the chance of lower GI adverse events as well as upper GI events. However, data from prospective studies regarding the rate of lower GI clinical events are not available. Coxibs decrease the rate of the upper GI clinical events, but information on their effect on lower GI clinical events also is not available. We assessed serious lower GI clinical events in a post hoc analysis of a prospective, double-blind outcomes study of rofecoxib vs. naproxen in 4267 patients followed for a median of 9 months. Rheumatoid arthritis patients \( \geq 50 \) years of age (or \( \geq 40 \) years if on steroids) were randomly assigned naproxen 500 mg bid or rofecoxib 50 mg qd. Patients were seen at 6 weeks, 4 months, 6 months, and q 6 months. Evaluation and treatment decisions were based on investigators' judgment rather than clinical situations. Serious lower GI clinical events (assessed blinded to treatment allocation) were defined as bleeding beyond the duodenum with \( >25 \) g/hour hemoglobin drop or hospitalization; and hospitalization for intestinal perforation, obstruction, ulceration, or diverticulitis. RESULTS (SEE TABLE): 35 patients had serious lower GI events: 24 with naproxen and 11 with rofecoxib. 27 patients had bleeding, 2 obstruction, 1 ulceration, 2 diverticulitis perforation, and 3 diverticulitis without gross perforation. In comparison, rates per 100 patient-years for all upper GI events and complicated upper GI events in this study were 4.5 and 1.4 for naproxen and 2.1 and 0.6 for rofecoxib. CONCLUSIONS: Serious lower GI clinical events occurred at an annualized incidence of 0.9% in patients taking the nonselective NSAID naproxen. This is 64% of the rate for complicated upper GI events with naproxen in the study. Serious lower GI clinical events were 54% lower with the selective COX-2 agent rofecoxib than with naproxen.

**Rates of Serious Lower GI Events per 100 Patients-Years in Patients Receiving Rofecoxib vs. Naproxen**

<table>
<thead>
<tr>
<th>Rofecoxib (N=4047)</th>
<th>Naproxen (N=4029)</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>1.1</td>
<td>2.00 (1.29 - 2.95)</td>
</tr>
</tbody>
</table>

* Relative risk of rofecoxib to naproxen from Cox model: \( p = 0.03 \)

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**Espinat-Barr Virus-Induced Gene 3 Deficiency Disrupts T Helper 2-Mediated Immune Responses in Experimental Colitis**

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The cytolytic Espinat-Barr virus-induced gene (EBI3) is an interleukin-12 p40 homologue expressed by macrophages and dendritic cells. Its function is unknown, however. To define the functional role of EBI3 we generated knockout mice in which the EBI3 gene was targeted by homologous recombination. EBI3-deficient mice were phenotypically normal but resistant to the induction of Th2 cytokines and immunopathology associated with oxazolone-induced colitis, a form of colitis thought to be mediated by Th2 type cytokines. In particular, EBI3 deficient mice showed no significant weight loss upon oxazolone sensitization and re-challenge, whereas wild-type mice showed a marked weight loss within 48 hours. Furthermore, there was a significant reduction in histologic signs of colitis in oxazolone-treated knockout mice compared to oxazolone-treated wild-type mice. Finally, we found that the induction of mucosal and systemic IL-4 responses seen in oxazolone-treated wild-type mice were suppressed in oxazolone-treated EBI3-deficient mice. In addition, the lack of IL-12 production, EBI3-deficient dendritic cells fail to promote a Th2 response to a model Th1 immunogen. These studies imply a critical regulatory role for EBI3 in the induction of a Th2-type immune response and for the development of Th2-mediated chronic intestinal inflammation in vivo.

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**Mutations in NOD2 Are Associated with Fibratostenosing Disease in Patients with Crohn’s Disease (CD)**


**Background:** The clinical manifestations of CD are diverse ranging from fibratostenosing disease of the small bowel to colon-predominant inflammation. These distinct clinical manifestations may be due to genetic, immunologic and microbial heterogeneity within CD. Mutations in the NOD2 gene have recently been described in CD and may alter innate immune responses in these patients. We hypothesized that mutations in the NOD2 gene may be associated with distinct phenotypic expressions of CD. Methods: Two separate CD clinical cohorts of consecutively-identified patients referred to an IBD center (n = 142 collected between 1993-96; n = 50 collected between 1990-2001) were analyzed. An IRB-approved protocol permitted genotype testing and clinical data abstraction. Genotyping was performed for three rare alleles of the NOD2 gene, R675W, G891R, and 890R, by the Taqman MGB system (Applied Biosystems, Foster City, CA, USA). CD was characterized as fibratostenosing, perianal fistulating, internal perforation or ulcerative colitis (UC)-like (UC-like; Gut 2000:47) by a panel of IBD physicians unaware of genotype information. Results: Univariate analysis revealed that the CD-associated NOD2 variants are significantly associated with fibratostenosing disease in the initial panel (p = 0.049) and second independent panel (p = 0.018) for the three allelic variants combined. When the two cohorts were analyzed together, the association between NOD2 variants and fibratostenosing disease was more significant (p = 0.003). These relationships were observed in both Jews and non-Jews within the cohorts. Approximately 40-50% of CD patients with fibratostenosing disease (Jews 52%, non-Jews 41%) have at least one of these rare alleles as compared to only 25% of CD patients without fibratostenosing disease (OR 2.6 and 95% CI 1.4-4.8). Of the three rare alleles, the frameshift mutation, 890R, demonstrated the greatest association with fibratostenosing disease (p for cohorts combined = 0.000). Conclusion: This is the first demonstration of a genotype-phenotype correlation in patients with CD. Further work needs to be done to determine the role of NOD2 variants. These data suggest that certain factors leading to fibratostenosing disease are related to specific impairments in the innate immune response regulated by NOD2. An understanding of this pathway may result in identification of host-microbial factors resulting in fibratostenosing disease.
Inflammatory Bowel Disease Is Associated with a Functional TNF Polymorphism That Affects an OCT1/NIK-8B Transcription Factor Interaction

Introduction: To assess genetic associations of TNF promoter variants in IBD and study the functional biology of associated variants. Methods: Association studies of the common TNF polymorphisms (-308I/R, -238G/A, -1031G/A, -857G/A, -862G/A). Two independent cohorts were used (set A, 457 IBD families: 294 Crohn disease (CD) trios, 252 ulcerative colitis (UC) trios; set B 130 IBD families and 278 healthy controls (HC)). CD analyses were further stratified by possession of NOD2 mutations. Functional studies of -857G/C: LPS stimulated whole blood TNF, ELISA: 46 healthy controls; monocyte nuclear extract promoter construct electro-mobility shift assays; in vitro GST pull-down assays of transcription factors OCT1, NF-kB, and deletion mutants; in vivo COS cell immune precipitation and COS cell luciferase reporter gene analysis. Results: TNF -857G/C was associated with IBD in both set A and replication in set B. We then assessed control and case variants of the OCT1 and NIK-8B trans-factor binding sites. Proportions homozygous for TNF-857G/C were IBD = 39.9% (P = 587 unrelated cases, P = 0.001). OCT1 NIK-8B negative 92.9% (241, P = 0.002); UC 92.1% (304, P = 0.01). Higher stimulated TNF production was seen in whole blood from TNF-857G/C homozygotes (P = 0.03) at 2 hours. The OCT1 transcription factor binding target TNF 1 and NIK-8B allele binding sites. DNA binding domains of OCT1 and NIK-8B pS5 translated in vitro and in vivo. OCT1 diminished NIK-8B induced reporter gene expression. Conclusion: The TNF-857G allele is associated with IBD, and TNF-857G OCT1 and NIK-8B homozygotes show higher TNF production. We have identified a molecular mode of action, through allele specific binding of OCT1 to the TNF promoter and interaction between OCT1 and NF-kB.

Role of the S531 Cytokine Gene Cluster Haploype (IBDS) in Japanese and British Inflammatory Bowel Disease: Evidence for Genetic Heterogeneity
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Introduction: Genetic variation in the S531 cytokine cluster has recently been linked to and strongly associated with Crohne’s disease (CD) in the Japanese population (IBDS locus, River et al., Nat Genet 2001). Although the disease gene has not been precisely characterised, the cluster contains the immune regulatory genes involved in Th1/Th2 responses. The role of the IBDS risk haplotype in ulcerative colitis (UC) remains unclear. (i) To determine the frequency of the S531 IBDS haplotypes in UC and Crohn’s disease in Japanese and British populations. (ii) To assess the association in the Japanese and UC populations. (iii) To assess genetic heterogeneity of the isolated markers (M-160, M-162) or epitope between IBDS and NOD2 variants. Methods: A genetic variant (GROR0064B) unique to the Japanese and the same frequency as the 258kb CD IBDS risk haplotype, was genotyped in 457 Japanese IBD families (253 UC, 249 CD trios) and Japanese cohorts (178 CD, 170 UC, 156 healthy controls). Family based and case-control association analyses were performed. CD analyses were sub-stratified by NOD2 status (carrier of Arg702Trp, Gly509Arg, Leu1007fsic). To assess genetic heterogeneity or epitasis, we used log-linear analyses in a ‘case-control study’ based on allele transmissions using the haplotype relative risk model. Results: No association was seen between the S531 risk haplotype and UC (Haplotype Transmission/untransmitted: 93/30, P = 0.24). Association was confirmed with CD, specifically in patients not carrying NOD2 mutations (110/87, P = 0.003)CD NOD2 carriers, 32/47, P = 0.3). In the UK population the haplotype plays a significantly lesser role in CD susceptibility than in the Japanese population (P = 0.92, OR 2.6, 95% CI 0.6-11.2, P = 0.24). Mean age at diagnosis in UK CD offspring was 22.4 years, similar results obtained for the TDT stratified above and below this age suggest age at diagnosis does not explain the weaker UK association. No interactions were seen between IBDS, Arg702Trp and Leu1007fsic. The IBDS risk haplotype was very rare in the Japanese population (<1% frequency) and stratified with the NOD2 association. Conclusion: The S531IBDS cytokine cluster risk haplotype does not influence susceptibility to UC, plays a lesser role in genetic susceptibility to CD than in the Japanese population; does not influence Japanese UC or CD. DO candidates demonstrate genetic heterogeneity.

Interleukin-10 Transduced T Lymphocytes Prevent Colitis in the SCID Transfer Model
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Regulatory CD4 + cells secreting the anti-inflammatory cytokine IL-10 play a key role in maintaining the immune balance in the intestinal mucosa. In this study we engineered primary CD4 + cells to express IL-10, and investigated the efficacy of this approach in protecting against experimental colitis. Spleen-derived CD4 + cells were transduced using a retroviral (MMLV) vector to simultaneously express human IL-10 and green fluorescent protein (GFP). The therapeutic benefit of CD4 + cells transduced with IL-10-GFP was studied in colitis, induced by transfer of CD45RAhiCD4 + cells, and in acute TNBS induced colitis. Transfected green GFP fluorescent cells were detected for at least 15 weeks in peripheral blood, spleens, colon and lymph nodes draining the intestinal recipient SCID mice. Human IL-10 was detected in draining lymph nodes, but not in the colon. In the TNBS model, inflammation was significantly reduced by transfer of IL-10-GFP CD4 + cells mean score: 0.72 ± 0.19) compared to untreated (2.33 ± 0.28) or control CD4 + cell treated (mean score: 2.57 ± 0.8). In conclusion, IL-10-GFP transduced CD45RAhiCD4 + cells lost the capacity to induce colitis (mean histological score 1.06) compared to untreated CD45RAhiCD4 + cells (mean score 5.1). In contrast, no therapeutic benefit was observed in TNBS-induced colitis. Taken together, peripheral CD4 + cells that were engineered to express IL-10 by retroviral transduction as regulatory cells in CD45RAhi-induced transfer colitis. This approach may induce long-term maintenance of mucosal immune homeostasis in Crohn’s disease.

Interleukin-10 Knockout Mice: A Model to Study Osteoporosis Associated with Intestinal Inflammation
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Background: Inflammatory bowel disease (IBD) is associated with decreased bone mineral density (BMD) and increased fracture risk. The pathophysiology of bone loss in IBD is poorly understood. RANKL, receptor activator of NF-κB, is a key mediator of osteoclasts, the bone resorbing cells. Our hypothesis was to study inflammatory cytokine gene expression and bone mass in RANKL knockout (UC) and wild type mice. Results: Both bone mass was measured by dual X-ray absorptiometry, bone structure by contact radiography, and trabecular bone mass by quantitative computed tomography (μCT). Bone RANKL mRNA expression was quantitated by Northern blot. T-cells isolated from spleen using positive selection with CD90 (Thy1) microabs from MLE mice (DRA, USA) were activated by ligation with anti-CD3e and -CD28 antibodies. An ELISA (R&D Systems, Minneapolis, MN) was used to measure their RANKL production. Results: Contact radiography did not show obvious bone abnormalities or fractures. Staining of the iliac crest at 8 weeks of age showed an age-dependent increase in bone mass, both in whole body BMD and vertebral trabecular bone mass in IL-10 mice with symptoms of enterocolitis. These parameters were unchanged in healthy IL-10 and wild type mice, suggesting that the IL-10 deficient state by itself does not influence bone mass. RANKL mRNA expression was again unchanged. Conclusion: IL-10 mice serve as a model to study bone loss associated with intestinal inflammation. In this model, increased RANKL production by activated T-cells may induce bone loss by increasing bone resorption. This was confirmed by bone histomorphometric analysis. Supported by the Patrick and Catherine Weldon Donoghue Medical Research Foundation, the University of Connecticut Center for Interdisciplinary Research in Women’s Health, and Nih grant DK75769 (ade).

Interleukin 10 Deficient Mice Are Detectible in Colonic Muc2 Synthesis Both before and after Induction of Colitis by Commensal Bacteria
Mireille K. Makkinke, Nicola J. Schwertworth, Maria Van Der Slaan, Hans A. Buller, Ryan Sandor, and Randa W. C. Ewing, Cleveland, OH

Colitis and IBD cause chronic low-grade inflammation in the colon, which then leads to an increase in the concentration of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β. Mucin 2 (MUC2) is produced by goblet cells and protects the mucosa against bacterial adherence. IL-10 deficiency has been shown to increase the expression of MUC2 in the colon. In this study, we measured the expression of MUC2 in IL-10 deficient mice before and after colitis induction by oral gavage with commensal bacteria. The results showed that IL-10 deficient mice had increased expression of MUC2 before and after colitis induction. This suggests that IL-10 deficiency may play a role in the development of colitis by increasing MUC2 expression.