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腸管粘膜マクロファージによる腸管ホメオスターシスとその破綻

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KEY WORDS

炎症性腸疾患，腸管マクロファージ，Interleukin-10，Interleukin-12，Interleukin-23

SUMMARY

腸管局所ではつねに多数の腸内細菌が存在しているため，腸管マクロファージ(Mφ)はそれら腸内細菌に対して過剰な免疫反応を引き起こさないよう，何らかの機構によって制御されていると考えられる．本研究により正常腸管 Mφ は IL-10 高産生の抑制性 Mφ であり，腸内細菌への過剰な免疫反応を制御していることが明らかになった．一方，炎症性腸疾患モデルである *IL-10* 遺伝子欠損マウスでは内因性 IL-10 の欠損のため腸管 Mφ が異常な分化を遂げ，腸内細菌に対し IL-12 や IL-23 といった Th1 誘導性のサイトカインを過剰産生することが明らかになった．このように，腸管 Mφ の腸内細菌に対する免疫制御機構の破綻が，腸内細菌に対する過剰な免疫応答を誘導し，炎症性腸疾患の病態に寄与していることが示唆された．

はじめに

消化管は消化，吸収，排泄をつかさどるだけでなく，複雑な gut associated lymphoid tissue (GALT) とよばれる免疫担当装置を形成している．さらに消化管には豊富な血管網や神経組織が迷路のように存在し，消化管ホルモンや神経ペプチドなどが生理機能を調節している．全消化管粘膜の表面積はテニスコート 1.5 面分にも及び，そこに 10^{14}個以上の腸内細菌が常在している．さらに，消化管は病原体や食餌抗原などの外来抗原にも恒常的に曝露されている．つまり，消化管は体内にありながらつねに外界と接している特殊な臓器といえる．

通常，免疫装置は外界からの侵入者に対して免疫反応を誘導することで外来抗原に対しすみやかに反応，処理することで生体を守っている．しかしながら，つねに食餌抗原や腸内細菌に曝されている腸管粘膜では，それらの抗原に対して過剰な免疫反応を誘導するのは好ましくなく，むしろ恒常性を保つため過剰な免疫反応を抑制的に制御する機構が存在すると考えられる．腸管上皮は構造的に微生物や抗原の侵入を防いでおり，さらにムチン，

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trefoil factor や抗菌ペプチドなどの分泌蛋白を産生し粘膜表面を守っている．しかし，これらの上皮細胞による防御にとどまらず，抑制性の免疫学的機構が存在していると思われる．実際，大腸粘膜をポリペクトミーで切除し粘膜を破壊してもわれわれは腸炎を発症することはない．また一過性に食あたりや感染性腸炎にかかることはあってもほとんどの場合は慢性化せず自然に沈静化する．この腸管の低反応性を説明する機序として腸管の自然免疫をつかさどるマクロファージの特殊性が明らかとなってきた．

炎症性腸疾患の病態に innate immunity は関与している

炎症性腸疾患（inflammatory bowel disease：IBD）は大きく潰瘍性大腸炎とクローン病の二疾患に区別される．これら2つの疾患は基本的に独立した疾患概念と考えられている．潰瘍性大腸炎では標的臓器は大腸のみであるのに対し，クローン病では小腸，大腸を含めた全消化管が標的となり，しばしば瘻孔を形成する．クローン病では腸管局所の免疫応答は type 1 helper T cell（Th 1）型にシフトしていることがわかっており，エフェクター細胞は腸管局所の $CD4^+T$ 細胞である．一方，潰瘍性大腸炎での局所の免疫応答の状態は報告により異なっており type 2 helper T cell（Th 2）型にシフトしているという報告もあるがコンセンサスは得られていない．潰瘍性大腸炎，クローン病ともにその病因はいまだ明らかにはなっていないが，遺伝素因，環境因子，免疫応答の異常が複雑に関与した多因子疾患であると考えられている．

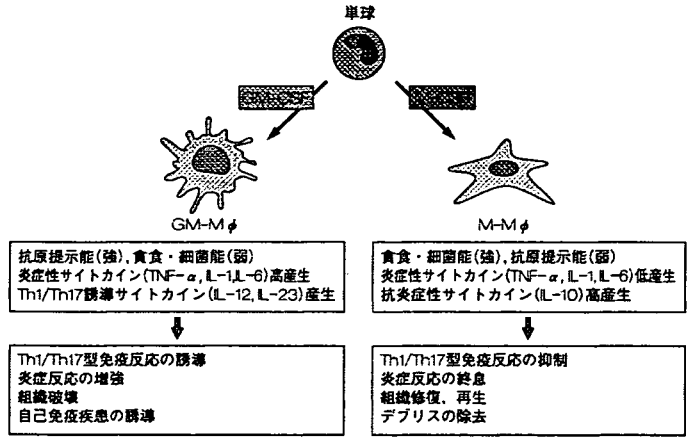
IBD の病因因子として近年最も注目されているのが腸内細菌の役割である．先に述べたように腸管では常在する腸内細菌に対してある種の免疫寛容が成立していると考えられるが，IBD ではおそらくこのバランスが破綻していると考えられる．実際にクローン病患者では anti-Saccharomyces cerevisiae antibodies（ASCAs），Omp C，I 2，flagellin に対する抗体 CBir 1 など酵母や腸内細菌に対する抗体価の上昇が認められ[1][2]，これらのことから食餌や腸内細菌などの何らかの外来抗原に対する異常な免疫応答が背景にあり，時として腸管局所のみならず全身の免疫系が活性化し自己抗原と交差反応することで関節炎などの自己免疫疾患に類似した症状も呈するのではないかとも考えられる．一方，遺伝的素因としては疾患関連遺伝子として *NOD 2* や *OCTN* などが同定されている．とくに NOD 2 は細胞質内 pathogen 認識分子として自然免疫（innate immunity）と IBD を関連づけるあらたな証拠として注目を浴び，IBD の基礎研究において innate immunity を再認識させるきっかけとなった[3][4]．

腸炎の発症には腸内細菌が関与する

これまで IBD モデルとして多くの遺伝子操作による自然発症腸炎モデルマウスが報告されてきた．これらのマウスは残念ながらヒト IBD を完全に反映しているとはいいがたいが，サイトカインやシグナル伝達に関与する分子など免疫に関与する分子の発現異常が慢性腸炎を引き起こすという事実は腸管における免疫機構の重要性を明らかにした点で重要な知見であった．しかしながらいずれのマウスモデルにおいてもなぜ発症するのか？という疑問は解決されないままである．ただ重要な点は多くのこれらマウスモデルは無菌状態（germ free）では発症しないということである．すなわち，たとえ免疫異常を有していても“フローラとの相互作用”＝“innate immunity”がなければ腸炎は起こらないのである．

腸内細菌は善玉か悪玉か？

前述したように IBD，とくにクローン病の病態には腸内細菌に対する過剰な免疫反応が関与していると考えられている．では腸内細菌はわれわれにとって炎症を引き起こす悪者なのであろうか？　近年，この疑問の答えとなるいくつかの研究が報告された．それらの研究において，腸内細菌の認識にかかわる Toll 様レセプター（Toll-like receptors：TLRs）やそのシグナル伝達分子である MyD 88 の欠損マウスではデキストラン硫酸ナトリウム（DSS）誘発腸炎が増悪すること，同様に腸内細菌の存在しない無菌マウスでは通常マウスにくらべ DSS 腸炎が増悪することが明らかになった[5][6]．すなわち正常の状態では腸内細菌と TLRs との相互作用は protective にはたらいている可能性が示唆される．このように腸内細菌の存在とそのバランスが腸の恒常性の維持にきわめて重



図❶ マクロファージの成長因子による機能の違い
マクロファージは成長因子の違いにより異なる2つのphenotypeへと分化する．GM-CSF誘導型のGM-Mφ（Mφ1ともよばれる）はIL-12, 23高産生な炎症惹起性マクロファージ，一方で，M-CSF誘導型のM-Mφ（Mφ2ともよばれる）はIL-10高産生でIL-12, 23は産生しない炎症抑制性のマクロファージである．腸管はM-CSF優位の組織であり，抑制性のM-Mφが分化し常在マクロファージを構築していると考えられる．

要であることは間違いがない．

腸管マクロファージは炎症抑制型マクロファージである

マクロファージは細菌などの外来抗原に対する自然免疫のおもな担当細胞であり感染防御において重要な役割を果たしている．しかしながら，腸管局所ではつねに多数の腸内細菌が存在しているため，マクロファージはそれら腸内細菌に対して過剰な免疫反応を引き起こさないように，何らかの機構によって免疫反応を制御していると考えられる．近年，マクロファージは分化誘導因子の違いにより相反する機能をもち異なる形態を示す2つのサブセットに分化することが明らかになった[7]．顆粒球マクロファージコロニー刺激因子（granulocyte-macrophage colony-stimulating factor：GM-CSF）により分化誘導されるGM型マクロファージは種々の炎症性サイトカインを高産生する炎症惹起型マクロファージであると考えられる．GM型マクロファージは強い抗原提示能を有し，獲得免疫の誘導に重要なインターロイキン（IL）-12やIL-23を産生することから，Th 1/Th 17型免疫反応の誘導に寄与していると考えられる．また，同時に腫瘍壊死因子（tumor necrosis factor：TNF）-αやIL-6といった炎症性サイトカインも強く産生し炎症反応を増強している．一方で，マクロファージコロニー刺激因子（macrophage colony-stimulating factor：M-CSF）により分化誘導されるM型マクロファージは抗原提示能が低く，逆に強い貪食・殺菌能をもつ．M型マクロファージはGM型と異なり細菌等の外来抗原刺激によりIL-12やIL-23などの炎症性サイトカインを産生せず，抑制性サイトカインであるIL-10を高産生する．すなわち，M型マクロファージはTh 1, Th 17型免疫反応に対して抑制的にはたらいていると考えられる．このように，これら異なる免疫応答を担うマクロファージサブセットがそれぞれの特性を発揮することで，生体の防御，そしてホメオスターシスの維持に重要な役割を果たしていると考えられる（図❶）．これらマクロファージサブセットの局在や腸炎における役割はこれまでまったくわかっていなかった．しかしながら，われわれ[8]の研究により，マウス正常腸管ではM-CSFが優位に発現していることが明らかになった．また，M-CSF欠損マウスである*op/op*マウスでは腸管マクロファージの分化が障害されているという事実からも，腸管はM-CSFの発

現が優位な組織であり，炎症抑制性であるM型マクロファージの分化の場であると考えられる[9]．実際にマウス腸管マクロファージは腸内細菌抗原刺激に対しTNF-αやIL-6などの急性反応性のサイトカインは産生するものの，決してTh 1，Th 17型免疫応答を引き起こすIL-12やIL-23を産生せず，むしろ抑制性サイトカインであるIL-10を高産生する抑制性のマクロファージであった[8]．またSmythiesら[10]はヒトの腸管マクロファージは細菌に対し貪食能を保ったままサイトカイン産生に関しては低応答となっていることを報告している．最近，腸管マクロファージが炎症抑制的にはたらいていることを裏づける報告として腸管マクロファージ欠損マウスではDSS誘導腸炎が増悪することが明らかになった[11]．このように正常な腸管マクロファージは腸内細菌に対し抑制性の免疫反応を誘導し，ホメオスターシスの維持にかかわっていると考えられる．

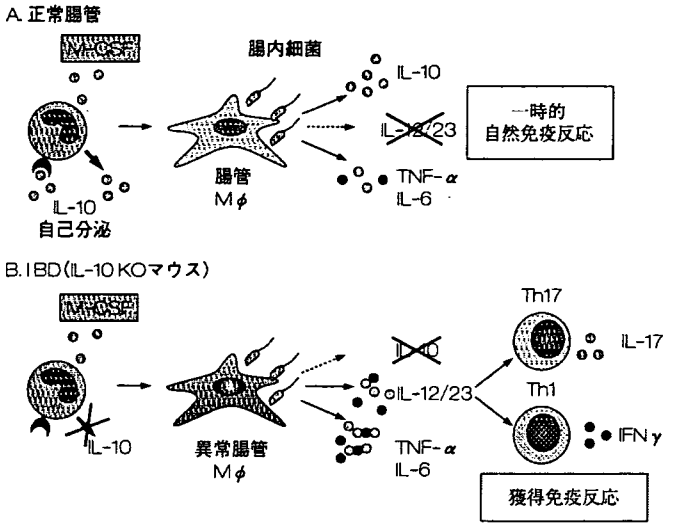
炎症性腸疾患（IBD）では腸管マクロファージ機能が破綻している

IBD，とくにクローン病においてその病態にマクロファージが重要な役割を果たしていることはいくつもの報告がある[12]．クローン病の腸管局所にはインターフェロン（IFN）γやIL-2産生に特徴づけられるTh 1型のCD 4$^+$T細胞が集積している．これらTh 1型のCD 4$^+$T細胞が産生するIFNγはマクロファージからのIL-12やIL-18などのサイトカインを産生し促し，IL-12，IL-18は逆にTh 1細胞を刺激することで炎症を持続させるサイクルが形成されると考えられる[13][14]．最近になり，いくつかのTh 1優位な疾患やそのモデルマウスにおいて，むしろIL-23/IL-17を主体とするTh 17免疫反応がより病的な意義が高いということが報告されはじめた[15]．実際にクローン病の腸管マクロファージからはIL-12のみならず，IL-23も高産生されるという報告もあり，クローン病の病態におけるIL-12/IL-23の役割が注目されている[16]．しかしながら，これらの報告は前述した腸管マクロファージ特有の炎症制御能とは一致しない．すなわちクローン病において腸管マクロファージは何らかの原因によりその免疫制御機能を失い，その結果，腸内細菌に対する過剰な免疫反応，IL-12/23産生に起因するTh 1/Th 17型獲得免疫反応の増強を引き起こしているのではないだろうか．

IBDモデル動物の1つである*IL-10*遺伝子欠損（knockout：KO）マウスはTh 1型の慢性腸炎を自然発症するモデルであり，クローン病の実験腸炎モデルとして広く用いられている[17]．IL-10 KOマウスのマクロファージや樹状細胞はナイーブT細胞やメモリーT細胞のTh 1反応を誘導する．また，腸管マクロファージを選択的に除去することで腸炎の発症が抑制されることから，本モデルにおいてマクロファージは炎症の主体をになっていると考えられる[18]．さらにIL-12 p 40とのダブルKOマウスやIL-12 p 40サブユニット抗体治療によりIL-10 KOマウスの腸炎発症が劇的に抑制されることから，クローン病と同様に，マクロファージからのIL-12/23産生が，本モデルの病態形成の鍵となっていると考えられる[19]．他のIBDモデル動物と同様，IL-10 KOマウスの腸炎発症，進展にも腸内細菌の存在が必須であることも報告されている[20]．しかしながら，これまで詳細なメカニズムについては不明であった．

われわれはマクロファージの腸内細菌認識機構に着目し，IL-10 KOマウスの骨髄単球由来GM型，M型マクロファージ，および腸管マクロファージの反応性について検討をおこなった．その結果，炎症性のGM型マクロファージではIL-12/23産生能に有意な差は認められなかったのに対し，本来抑制性にはたらいている骨髄由来M型マクロファージ，腸管マクロファージにおいて腸内細菌である*Escherichia coli*や*Enterococcus faecalis*加熱死菌抗原刺激により過剰なIL-12，IL-23の産生が認められた[8]．

つぎに，なぜIL-10の欠損はM-CSF誘導性のマクロファージにのみ強く影響するのだろうかという疑問がもちあがる．過去の研究により，単球をM-CSFで刺激するとIL-10が誘導されることがわかっている[21]．しかしながらGM-CSFにはこのようなIL-10誘導能は認められない．すなわち，M-CSFにより分化誘導される抑制性のマクロファージ（腸管マクロファージも含めて）の機能的成熟には分化時にM-CSFにより誘導されるIL-10の自己分泌刺激が必要なのではないだろうか．この仮説を証明するために，われわれはIL-10 KO M型マクロ



図❷ 腸管マクロファージ分化異常による免疫抑制能の破綻

A. 正常腸管マクロファージは腸管に存在するM-CSF依存的に分化する．この時M-CSFにより誘導されるIL-10の刺激を受け，腸管マクロファージは腸内細菌に対しTNF-αやIL-6などの急性反応性のサイトカインは産生するものの，獲得免疫応答を誘導するIL-12やIL-23を産生せず，むしろ抑制性サイトカインであるIL-10を高産生する抑制性の機能を獲得すると考えられる．

B. IL-10 KOマウス由来腸管マクロファージはその抑制機能が破綻し，腸内細菌に対しTNF-αやIL-6のみならずIL-12やIL-23を過剰産生し，Th 1型やTh 17型の獲得免疫反応を誘導していると考えられる．

ファージの分化時にIL-10を加え分化実験をおこなった．刺激時にはIL-10は完全に除去し，検出できるレベルにないことを確認した．結果，IL-10を分化時に加えたマクロファージではIL-10産生能がないにもかかわらずIL-12, IL-23の過剰産生は抑制された．本結果よりわれわれ[8]は，通常下では分化段階で内因性のIL-10によりマクロファージによるIL-12誘導機構(つまりはTh 1優位な獲得免疫の誘導)は負に制御されているが，IL-10欠損下ではIL-12の抑制機構が破綻し，その結果，腸内細菌認識により過剰なIL-12が産生され，Th 1優位な腸炎を引き起こすことを明らかにした（図❷）．

興味深いことに，腸内細菌刺激によるIL-12/23の過剰産生はマクロファージによる貪食を阻害することで抑制された(未発表データ)．また，リポポリサッカライド(LPS)，ペプチドグリカンなどのpathogen associated molecular patterns (PAMPs) 刺激ではIL-10 KOマウスにおいても過剰なIL-12/23産生は認められなかった[8]．詳細なメカニズムは現在検討中であるが，貪食された細菌の認識にはTLR以外の細胞内認識機構を介している可能性がある．前述のクローン病疾患関連遺伝子である細胞内細菌認識レセプターNOD 2/CARD 15などの知見とあわせて考えても非常に興味深い．

おわりに

以上のように，腸管局所ではマクロファージは貪食，殺菌能は有しているがIL-12, IL-23などのTh 1, Th 17誘導性サイトカインを産生せず，抑制性サイトカインであるIL-10を高産生する抑制性の性質に分化している．つまり，正常腸管マクロファージは，外来の病原体に対する自然免疫反応を介した防御能を維持しながら，食餌抗原や腸内細菌に対する過剰な免疫反応を制御していると考えられる．このように消化管は非常に複雑で精密な

仕組みでホメオスターシスを保っており，その破綻がIBDという特殊な慢性持続炎症を引き起こすものと考えられる．

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Psychological aspects of inflammatory bowel disease

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Key words: inflammatory bowel disease, ulcerative colitis, irritable bowel syndrome, psychological stress, enteric nervous system, brain-gut axis

Introduction

Psychological stress has been described as "a process in which environmental demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place persons at risk for disease."[1]

Psychological stress is widely believed to play a major role in functional gastrointestinal disorders, especially irritable bowel syndrome. There is a long history of observations suggesting that psychological stress contributes to the course of inflammatory bowel disease (IBD). The chronic medical conditions characterizing IBD, chronic diarrhea, bloody stools, abdominal pain, weight loss, malnutrition, and weakness, seem to be exacerbated by physiological and psychological stress. From this viewpoint, we often see an overlap of pathophysiology between IBD and irritable bowel syndrome (IBS). Furthermore, recent studies on IBS have demonstrated that dysregulation of the immune system and its interaction with bacteria/flora may contribute to IBS pathophysiology, just as with IBD. Here, we review the role of psychological stress in IBD, including our current preliminary observations of patients with ulcerative colitis (UC), and the possibility of an overlap in pathophysiology between IBD and IBS.

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The influence of psychological stress on gastrointestinal tract homeostasis through corticotrophin-releasing factor, a key player in the brain–gut axis

Stress can be defined as any threat to an organism's homeostasis.[2] The function of the stress response is to maintain both psychological and physiological homeostasis. Stress stimulates the release of corticotrophin-releasing factor (CRF) from the hypothalamus, causing the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. Stress finally stimulates secretion of cortisol from the adrenal cortex, and it also directly activates the autonomic nervous system. Stimulation of the sympathetic nervous system in response to stress causes the release of adrenaline and noradrenaline from the adrenal medulla. The autonomic nervous system also directly affects all nerves of the gut, that is, the enteric nervous system (ENS). The ENS contains around 100 million neurons and provides a highly systematic neural network in the gut. Thus, brain and ENS together make up a network termed the brain–gut axis[3,4] (Fig. 1). While central CRF regulates the ACTH-cortisol system, peripheral CRF directly induces alteration of gastrointestinal (GI) motility. Endogenous CRF mediates the stress-induced inhibition of the upper GI tract and the stimulation of colonic motility. The inhibition of gastric emptying by CRF may be through CRF-2 receptor signaling, while CRF-1 receptors are involved in colonic motility in response to stress.[5] Consistent with this is that peripheral administration of CRF antagonist affects colonic and gastric motility.[6] Endogenous serotonin (5-hydroxytryptamine, 5-HT) released in response to stress seems to be involved the alteration of colonic motility by stress-induced CRF through 5HT-3 receptors. Further, CRF is thought to have the potential to change the production of several cytokines[7,8] and the function of immune cells, including lymphocytes and NK cells.[9] Interestingly, it has been reported that CRF contributes to the

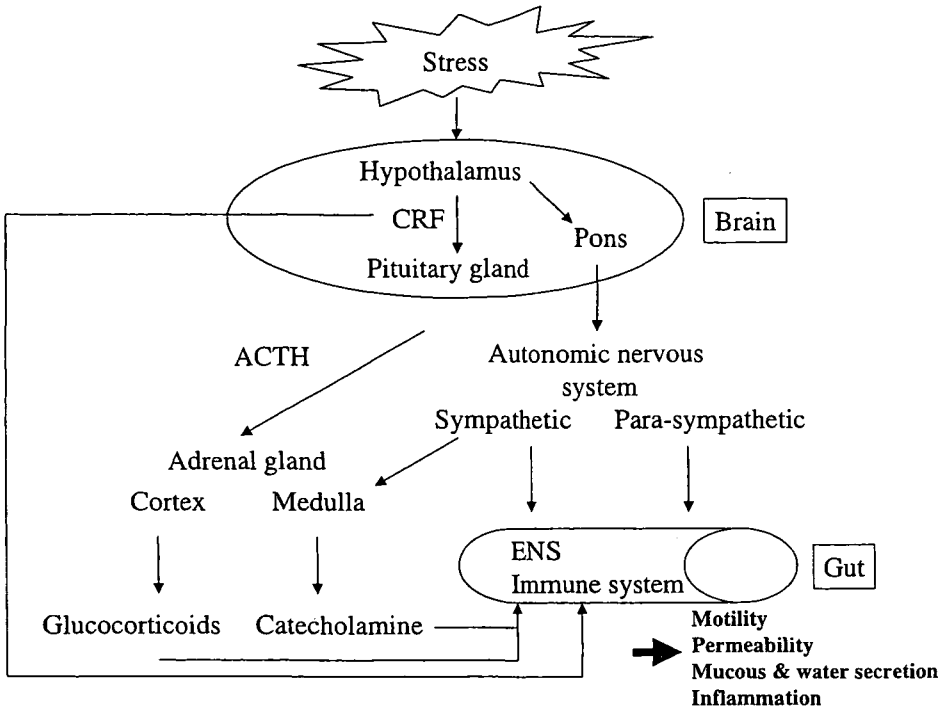

Fig. 1. Schema of the brain–gut axis. *CRF*, corticotrophin-releasing factor; *ENS*, enteric nervous system; *ACTH*, adrenocorticotropic hormone

pathophysiology of human UC. CRF levels are increased in lamina propria mononuclear cells from patients with active UC.[10,11]

Thus, both central and peripheral CRF systems are stimulated by stress and may have the potential to regulate gut homeostasis and so influence IBD pathophysiology.

Psychological stress alters intestinal barrier functions and water secretion

In several experiments using animal models, it has been demonstrated that stress can increase intestinal mucosal permeability and alter bacteria–host interactions. Restraint stress in rats increases jejunal and colonic mucosal permeability,[12,13] possibly by altering the cholinergic nervous system[14] and mucosal mast cell functions.[15] Catecholamine induced by stress also increases bacterial adhesion to intestinal mucosa.[16] Stress in animal models has also been shown to increase water and mucous secretions modulated by the cholinergic nervous system or mast cells, causing increased colonic motility and defecation, similar to observations in human IBS or IBD.[17–20] These observations of increases in stress-induced water and mucous secretions in animal stress models are likely to be consistent with human IBD pathophysiology.

Psychological stress in the course of human IBD

In many clinical trials involving novel therapies for IBD, a high rate (20%–40%) of improvement is often observed in the placebo group. These high placebo effects suggest to us that the IBD course and symptoms are often influenced by psychological conditions. Moser et al.[21] reported that 74% of IBD patients believe that psychological factors contribute to the course of their disease, which is significantly more than is found for other medical outpatients. However, scientific evidence in support of the contribution of psychological stress to IBD pathophysiology is inconsistent. Further, the efficacy of reduction therapy for psychological stress in IBD patients is controversial.

An initial question is, does psychological stress affect the onset of IBD? Li et al.[22] reported a follow-up study in Denmark of parents who had lost a child. They studied the onset of IBD in 21062 parents who had lost a child from 1980 to 1996 in Denmark compared with 293745 family-structure-matched parents. The relative risk of a first hospitalization for Crohn's disease (CD) was 0.97, and 1.01 for UC. Based on these results, they concluded that there was a negative association between psychological stress and the development of IBD in young to middle-aged adults. While studies of the role of psychological stress in the onset of IBD are relatively rare, a number of clinical studies investigate role of psychological stress in the clinical course of IBD, for example, in inducing relapse or worsening. Murray[23] first reported the contribution of psychological stress to

UC in 1930; however, it was not fully shown scientifically. In the 1990s, North et al.[24,25] reviewed the contribution of psychological stress to IBD pathophysiology.[24,25] However, as noted by Maunder,[26] the contribution of psychological stress to human IBD remains controversial. His review assessed several prospective studies of stress, depression, and IBD course. However, the patient populations were not matched in these studies; two studied only pure UC cases,[27,28] two only pure CD cases,[29,30] while five comprised cases of both UC and CD.[32-35] Baselines of disease activity and observation periods also varied. As well, outcomes of studies [relapse, Crohn's Disease Activity Index (CDAI), or symptom diary] differed among studies. Thus, studies that are not cohesively designed can lead to confusing results. In UC, Bitton et al.[28] reported that the odds ratio of relapse by life events was not very high (1.26 per event). Levenstein et al.[27] demonstrated a failure to determine a relationship between life events and relapse.

On the other hand, Mawdsley et al.[36] reported that acute psychological stress induced systemic and mucosal cytokine release in patients with inactive UC.

To assess of the effect of psychological stress for relapse of UC, we administered a multiple-choice questionnaire to 212 patients with UC. To focus on the effect of psychological stress for a relapse of UC and to adjust the baseline, we selected 160 patients with UC who in the past 3 years had had no event of macroscopic bloody diarrhea lasting 1 month (Fig. 2). Among these patients, 138 patients had had episodes of relapse in the most recent 3 years. Twenty-two patients remained in remission. As in previous reports, the most prominent factor involved in relapse by patients was psychological stress (66.2%). Interestingly, we found a seasonal fluctuation of relapse in these patients, suggesting an affect of life events on relapse (Fig. 3). Another question relates to the prognosis of relapsing patients who claim psychological stress as a cause of their relapse. The prognosis of patients with relapse who selected "positive psychological stress" on the questionnaire was not very severe. Indeed, 63.4% of the patients claiming positive psychological stress improved without additional medication or with treatment only involving an enema or a suppository (Fig. 4). As in Maunder's review,[26] it is quite difficult to find direct evidence of a correlation between relapse and psychological stress. Although many patients selected psychological stress as a risk factor for their relapse in this multiple-choice questionnaire, they also selected other factors, such as sleeplessness, physiological distress, and excessive eating or drinking. As in many earlier human studies, it was also hard in this study to assess the effect of pure psychological stress on IBD. Several reasons that studies of the role of stress in IBD are difficult and vague are (1) IBD itself may be a basket disease entity comprising multiple features; (2) there are no adequate methods to measure psychological stress (scoring of life events?); (3) sensitivity to psychological stress may be different among patients; (4)

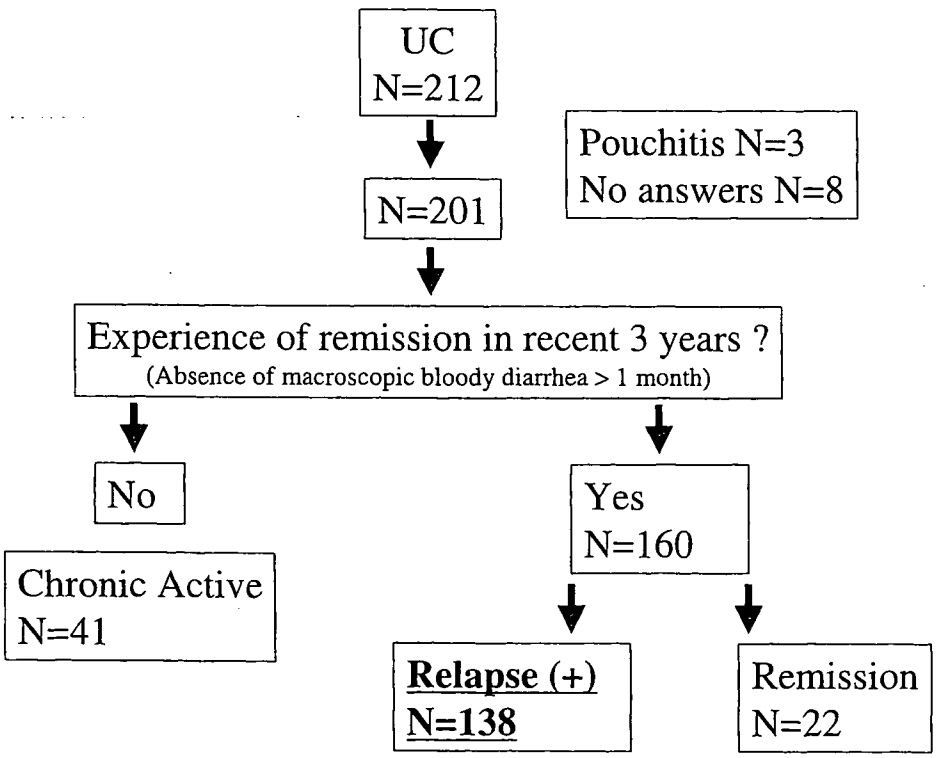

Fig. 2. Multiple-choice questionnaire regarding triggers of a relapse of ulcerative colitis (*UC*). The multiple-choice questionnaire was administered to 212 patients with UC. Of these, 138 patients with UC who in the past 3 years had had no macroscopic bloody diarrhea lasting 1 month were analyzed for the assessment of the role of psychological stress in the relapse of UC

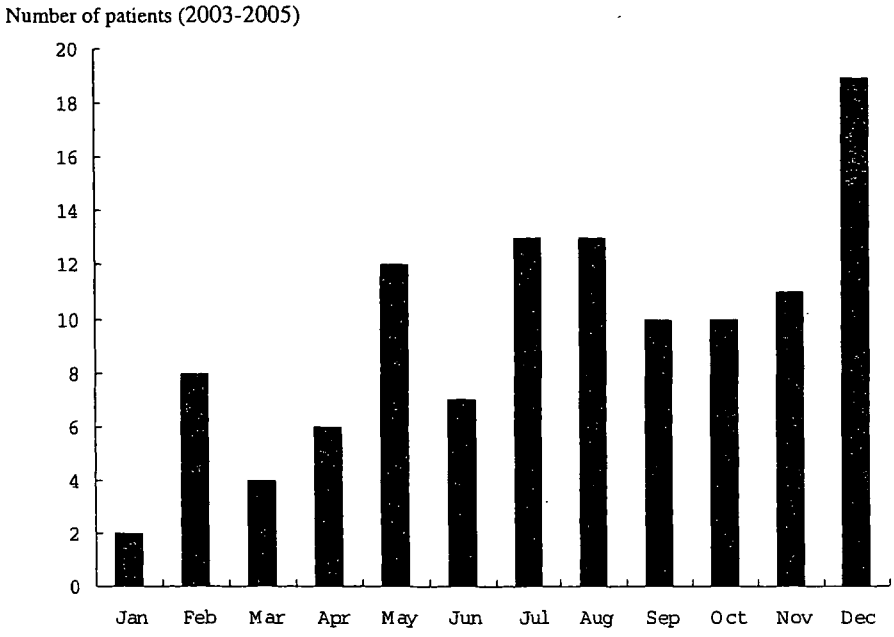

Fig. 3. Seasonal fluctuations of relapses in patients with UC. Results are based on a multiple-choice questionnaire. The *Y* axis shows the number of relapse patients out of 138 patients

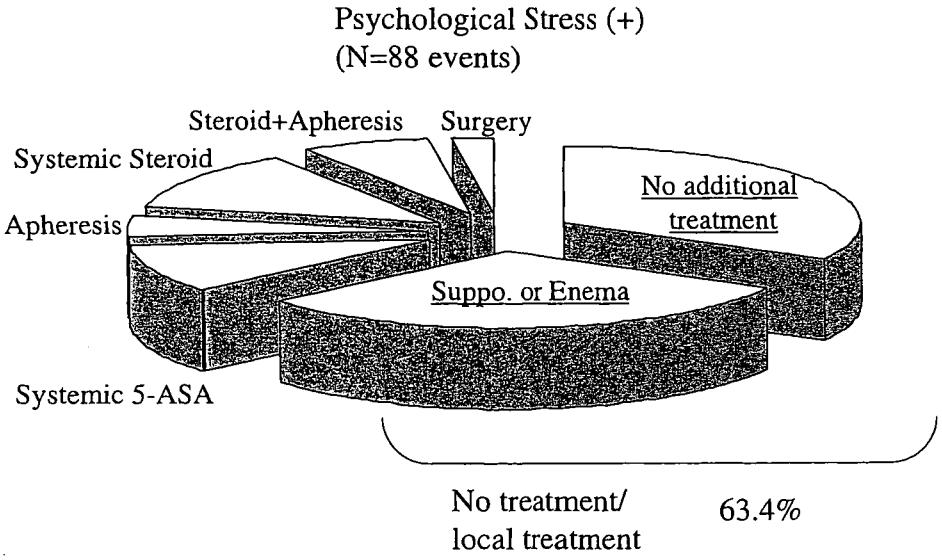

Fig. 4. Prognosis of patients with relapse who implicated psychological stress as a cause of the relapse ("psychological stress positive"). Suppo., suppository; 5-ASA, 5-aminosalicylic acid

there are difficulties in completely neglecting other risk factors, including smoking in CD and use of nonsteroidal anti-inflammatory drugs, in the studies; and (5) it is difficult to design a long-term prospective observation study. Well-organized prospective studies (not a mixture of CD and UC cases, an adjusted baseline, a sufficient observation period, adequate outcomes, and adequate methods for the measurement of stress) are necessary to provide a clearer answer to this historical issue.

Are there overlaps between IBD and IBS in pathophysiology?

IBS is a common chronic functional bowel disorder characterized by intermittent or continuous abdominal pain and alterations in bowel patterns. At this time, IBS is a diagnosis of exclusion, and there are no serological markers or specific pathological findings. For the clinical diagnosis of IBS, the Rome II criteria are widely used.[37,38] It has been reported that the colonic motor response to various psychological and physical stressors is increased in IBS patients.[39–42] While the etiology of

IBS remains unclear, many studies have suggested an overlap between IBS and psychiatric disorders such as anxiety, depression, and somatization disorders.[43]

Thus, while IBS has in recent years been considered to have symptoms caused mainly by unbalanced stress-induced psychological conditions, altered bacteria–host interactions and the mucosal immune system have been suggested to have a role in the mechanisms of action of IBS. Khan and Collins[44] demonstrated a relationship between the immune and motor systems using animal models. They demonstrated that the T helper 2-type immune response is critical for producing alterations in infection-induced intestinal muscle function. Collins[45] presented the case for an immunological basis for IBS. Several other studies have demonstrated activation of the mucosal immune system and active inflammation in IBS.[46,47] Ohman et al.[48] reported that peripheral α4β7-positive T cells, which may be localized to the intestinal mucosa and play an important role as the pathogenic T cells in IBD pathophysiology, are increased in IBS patients. This population of T cells has already been identified as the therapeutic target in CD patients using anti-α4 integrin monoclonal antibody.[49] Furthermore, because IBS is diagnosed by clinical criteria without any lower intestinal findings, patients with microscopic colitis, which is characterized by pathological lymphocyte infiltration into the intestinal mucosa, are often misdiagnosed.

Other current issues regarding IBS include postinfectious IBS and the role of host–bacteria interactions in the pathophysiology of IBS. Since the 1990s, many reports have demonstrated the possibility that an entity called "postinfectious IBS" may exist as a subgroup of IBS. McKendrick et al.[50] reported IBS arising in patients after *Salmonella* infection. Several reports have demonstrated that bacterial gastroenteritis might be a risk factor for the development of IBS.[51–56] Based on these observations, several therapeutic trials for IBS using antibiotics or probiotics have been reported. O'Mahony et al.[57] demonstrate that *Bifidobacterium infantis* 35624 can reduce symptoms in IBS patients. Sharara et al.[58] demonstrate the efficacy of rifaximin in patients with abdominal bloating and flatulence. Because these therapeutic strategies for IBS remain controversial, larger well-designed studies are necessary.

Thus, IBS, as it is now diagnosed, is possibly part of a global disorder; therefore, it is not unreasonable to postulate that there is some overlap of pathophysiology between IBS and IBD. Although several genetic backgrounds have been demonstrated in IBD, including NOD2,[59,60] OCTN,[61] and TNFSF15,[62] they are not correlated with the patterns of IBS. These findings suggest to us that the genetic background is more important in the pathophysiology of IBD than in that of IBS. While psychotherapeutic interventions have increasingly been used to treat refractory IBS, the efficacy of these therapies on IBD has not been scientifically demonstrated, and their effects in IBD treatment remain controversial. In this regard, psychological factors seem to contribute more strongly to IBS than to IBD (Table 1). Thus, although some historical observations suggest an overlap in pathophysiology between IBS and IBD, further studies in human and animal models are necessary to reach a better understanding. In particular, because both IBS and IBD may be part of a global disease entity, these disorders need to be classified into subclasses and the pathophysiological mechanisms in each group analyzed (e.g., high genetic background IBD, psychological factor-dependent IBD, IBS caused by immune disorders, responders in IBS/IBD to antibiotics or probiotics therapy). Such investigations should help in establishing patient-specific therapeutic strategies.

Table 1. Are there any overlap inn pathophysiology between IBD and IBS? (or just overlap of letters "IB"?)

	IBD	IBS
Genetic background	○	?
Immunological dysregulation	○	△
Bacteria–host interaction	○	△
Food	△	?
Dysregulation of motility	△	○
Mucosal permeability	△	△
Psychological stress	?	○

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome
○, strong; △, controversial; ?, unknown

Conclusions

Since the 1930s, psychological stress has been thought to contribute to IBD pathophysiology. However, the scientific evidence is not consistent. Moreover, there is not sufficient scientific evidence to determine whether an overlap between IBD and IBS pathophysiology exists. However, several human studies and studies in animal models have yielded important clues regarding the historical question, "What is the role of psychological stress in IBD." To obtain a definitive answer, further well-organized prospective studies using scientific methodology to measure psychological stress are necessary.

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Review

Novel pathophysiological concepts of inflammatory bowel disease

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Key words: inflammatory bowel disease, Crohn's disease, ulcerative colitis

Introduction

Gut-associated lymphoid tissue (GALT) harbors more than 80% of the total lymphoid tissue in the body, and intestinal inflammation is caused and/or maintained by abnormal immune responses to foods or microbes in GALT (Fig. 1). Although the pathogenesis of inflammatory bowel disease (IBD) is still unknown, significant progress and new insights have been gained through a wide variety of analyses consisting of genetic factors, environmental factors, and immunological abnormalities (Fig. 2). The unraveling of immunopathogenic mechanisms has been critical to the discovery of new therapeutic targets, which has, in turn, driven the development of new biological therapies. Recently, several excellent reviews have focused on IBD pathophysiology. Bouma and Strober[1] discussed recent advances in understanding the induction and regulation of mucosal inflammation, highlighting the role of mucosal T cells. Recent advances in understanding the function of mucosal T cells were also the focus of an article by Watanabe et al.,[2] and Gordon et al.[3] contributed a very detailed chapter on cytokines, chemokines, and growth factors in the pathogenesis of IBD to a book on cytokines and chemokines in autoimmune disease. Innate immunity and the role of intestinal bacteria were excellently reviewed by Macdonald and Montelone[4] and Elson et al.[5] The genetics of IBD have been reviewed by Annese et al.[6] A good general overview has been published by Rogler.[7] Herein, the updated pathophysiological concepts of IBD are reviewed.

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Genetic factors

It is very clear that genetic factors play an important role in the pathogenesis of IBD and in both Crohn's disease (CD) and ulcerative colitis (UC). Epidemiological studies in monozygotic and dizygotic twins, as well as family studies, have indicated that genetic factors may be more important in CD than in UC.

The search for susceptibility genes had its first success in 1996, when the first susceptibility locus for CD was identified in the pericentromeric region of chromosome 16, which was called IBD1. In 2001 a caspase recruitment domain-containing protein, CARD15/NOD2, was found to be mutated in 20%–30% of CD patients, establishing a proof of principle for the "genetic concept" of IBD pathophysiology. Multiple mutations in the CARD15/NOD2 gene have been identified (Fig. 3), three of which have been shown to be independently associated with CD (arg702trp, gly908arg, and leu1007fsinsC).[8] These three variants confer a 15%–20% attributable population risk among cases of familial CD. The relevance of CARD15/NOD2 for the etiology of CD was confirmed in a number of subsequent studies.[9,10] CARD15/NOD2 mutations are associated with ileal disease, earlier age of disease onset, and stricturing disease.[11] In contrast, a few articles support data that CARD15/NOD2 mutations do not play a role in the etiology of CD in Asia[12] (see Fig. 3). However, there are several abnormalities in genetic factors of Japanese IBD patients, including the HLA-DR regions.[13,14] Interestingly, most of these abnormalities are not found in Western countries. In addition, healthy homozygous carriers of the 3020insC frameshift mutation have been described,[15] indicating that CARD15/NOD2 mutations are not the sole determinant of CD and that environmental factors also play an important role. Although epidemiological data concerning CARD15/NOD2 are rather clear and have been con-

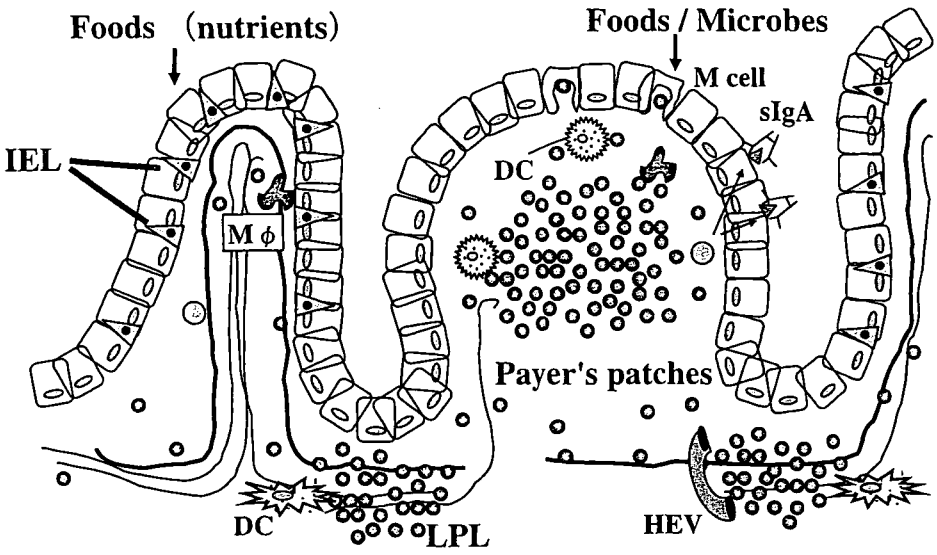


Fig. 1. Mechanism of gut-associated lymphoid tissue (GALT). Intestinal mucosa has a specific immunological apparatus, GALT, and plays a role in the defense system against microorganisms or food antigens from the luminal side

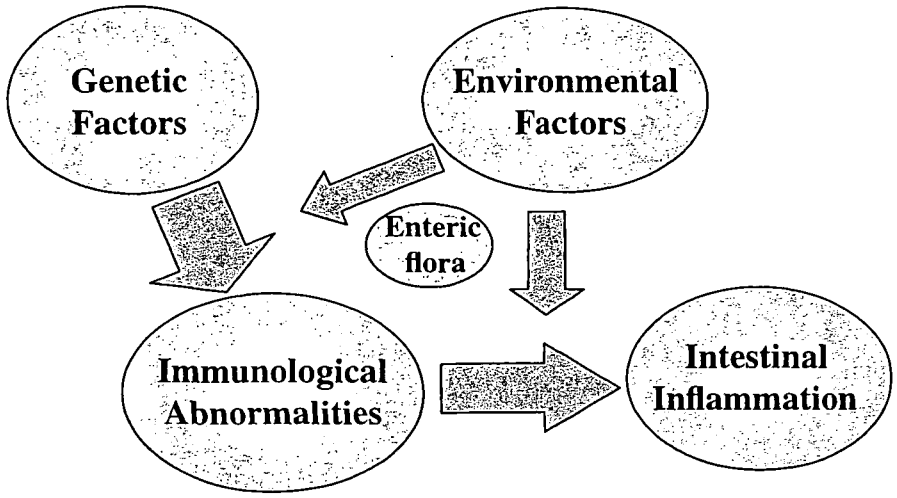


Fig. 2. General background of inflammatory bowel disease (IBD)

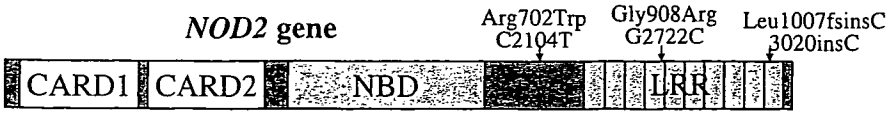
firmed, the pathway of how these mutations cause CD is less clear.

By immunohistochemistry, in situ hybridization, and reverse transcription-polymerase chain reaction, Berrebi et al.[16] showed that CARD15/NOD2 was present only in the cytoplasm of macrophages in the normal colon. Increased CARD15 expression was detected in intestinal epithelial cells (IECs) and macrophages in CD lesions. A role for Paneth cells in CARD15/NOD2-induced pathophysiology is supported by data showing CARD15/NOD2 mRNA-enriched Paneth cells in CD mucosa.[17] Expression of CARD15/NOD2 by IEC has also been shown by Hisamatsu et al.[18] They provide evidence that CARD15/NOD2 mRNA and protein were upregulated by tumor necrosis factor (TNF)-α in SW480 cells.[18]

Environmental factors

Among the environmental factors, food intake seems to be one of the most important factors that affects the pathophysiology of IBD (Fig. 4). Sakamoto et al.[19] analyzed what kind of foods were risk factors for IBD, comparing food habits of the patients with those of healthy controls at the same age. They found that intake of fast foods containing large amounts of fat and sugar-rich foods may accelerate the development of CD. Another study demonstrated that long-chain fatty acids are more irritable for intestinal inflammation than medium-chain fatty acids.[20] In most Western countries, sugar-rich foods have also been recognized as one of the risk factors for CD.

Intestinal flora and mucosal defense systems are also playing major key roles. The evidence that bacteria play



	Population	Disease	Allele frequencies of the mutations (%) C2104T	G2772C	3020insC
Ogura et al (Nature 2001)	American	NL	4.0	1.7	4.0
		CD	10.8	4.3	8.4
Hugot et al (Nature 2001)	European	NL	4	1	2
		UC	3	0	1
		CD	11	6	12
Hampe et al (Lancet 2001)	German and British	NL	N.D.	N.D.	4.8
		UC	N.D.	N.D.	3.1
		CD	N.D.	N.D.	16.0
Inoue、Hibi et al (Gastroenterology 2002)	Japanese	NL(n=272)	0	0	0
		UC(n=292)	0	0	0
		CD(n=350)	0	0	0
Leong et al (Aliment Pharma-Col Ther 2003)	Chinese	NL(n=70)	0	0	0
		UC(n=63)	0	0	0
		CD(n=65)	0	0	0
Croucher et al (Eur J Hum Genet 2003)	Korean	NL(n=232)	0	0	0
		CD(n=252)	0	0	0

Fig. 3. Crohn's disease (CD) and *NOD2* gene. Three variants in the coding region of the *NOD2* gene, located on chromosome 16q12, are associated with CD

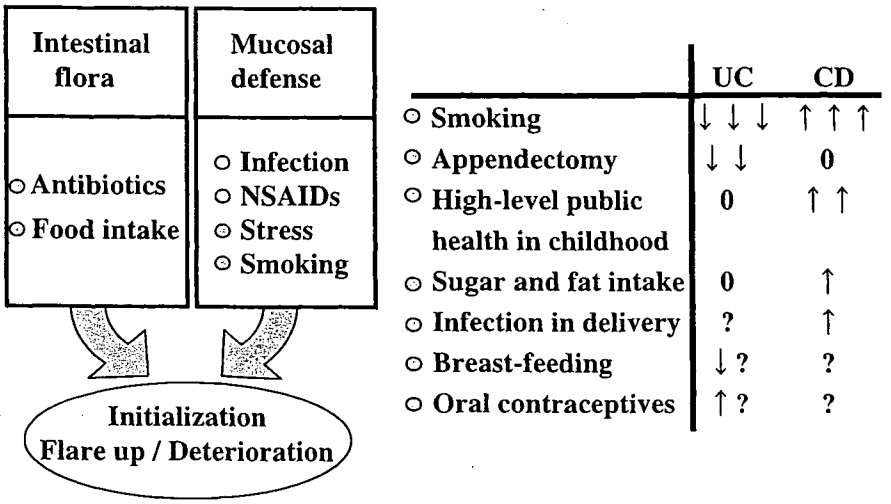


Fig. 4. Environmental factors that affect the initialization/flare-up and deterioration of IBD

a major role in the initiation and perpetuation of intestinal inflammation comes from studies with germfree animal models, a condition under which these animals do not develop intestinal inflammation, in contrast to specific pathogen-free rodents.[21] In colonic lesions of CD patients, adherent-invasive *Escherichia coli* have been found. In addition, an increased bacterial translocation into deeper layers of the mucosa has been described in CD patients, which could be of pathophysiological relevance. *E. coli* Nissle has been proved to have therapeutic potential in IBD. The mechanism could be an inhibition of the adherence and invasion of pathogenic *E. coli*,[22] which further supports a role of bacterial translocation into the mucosa in the pathogenesis of CD. In fact, fecal bacterial composition is altered in CD patients compared with healthy control subjects.[23] A role for certain bacteria in the pathogenesis of IBD is further supported by the positive effects of probiotic bacteria on intestinal inflammation and secretion of proinflammatory cytokines.[24] A lysate of *E. coli* ameliorates disease in a colitis model.[25] An increased bacterial invasion into the mucosa could be caused by ineffective innate responses such as mutated and defective CARD15/NOD2 or NF-κB protein. On the other hand, impaired or defective protection mechanisms of the mucosa could be involved. Direct mucosal protection is mediated by molecules such as mucins, trefoil peptides, or defensins. A deficiency in these molecules could cause a breakdown of mucosal protection.[26]

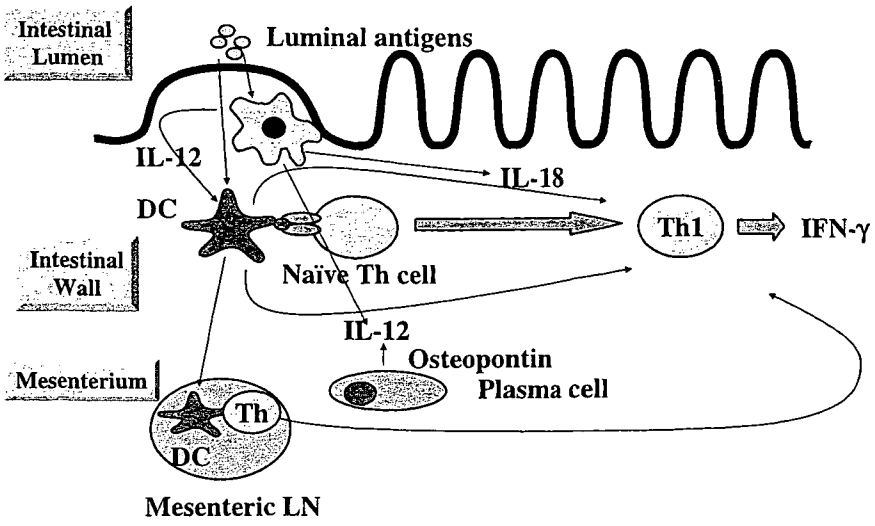


Fig. 5. Pathophysiology of Crohn's disease (CD)

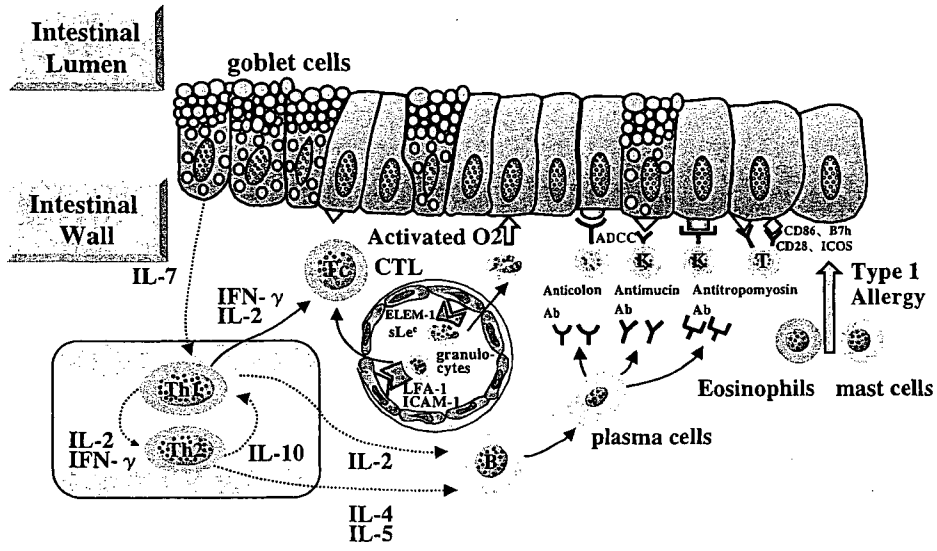


Fig. 6. Pathophysiology of ulcerative colitis (UC)

Prebiotics are nondigestible food constituents that benefit the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon. Some examples of prebiotics are dietary fiber and some types of oligosaccharides. Intake of prebiotics can significantly alter the colonic microflora by increasing the populations of certain bacteria and thereby quantitatively changing the composition of the microflora. These alterations may act beneficially, in part, by causing a luminal increase in short-chain fatty acids (SCFAs), which are important nutrients for the intestine and inducers of an acidic environment. Butyrate, the most important SCFA, is a potent antiinflammatory factor in local chemokine secretion.[27] Clinical usefulness of prebiotics for treatment of IBD patients has been reported.[28,29]

Several studies have addressed the impact of family history of smoking in Asia as well as in Western countries. In Japanese patients, smoking showed a protective effect against UC,[30] while no relationship between smoking and severity of UC was detected in a large population of Chinese patients.[31] Among Chinese patients, exsmokers, but not current smokers or previous and current smokers combined, were at greater risk of developing CD.[32]

The protective effect of appendectomy in UC was also evaluated in a large multicenter case-control study of Japanese patients.[33] As found in European and American studies, the results showed that appendectomy had a negative association with development of UC, particularly when performed in younger patients. In a Korean study, appendectomy was also found to be protective against UC. These studies suggest that changes in environmental factors may be one of the important causes for the gradual increase of IBD patients in Asia.

Immunological abnormalities

Crohn's disease (CD) results from an excessive and persistent CD4 T-helper cell type 1 (Th1) in the gut mucosa[34] (Fig. 5). Tissue from the gut of patients with CD contains abundant transcripts for interferon (IFN)-γ, and isolated mucosal T-cells secrete large amounts of INF-γ.[35] Production of interleukin (IL)-12, one of the key cytokines involved in Th1 polarization and differentiation, is markedly increased in patients with CD.[36] The increased expression of Th1 cytokines in CD is associated with T-bet, an IFN-γ-inducible novel member of the T-box family of transcription factors.[37] IL-18 also drives Th1 cell differentiation, activates the transcription factors AP-1 and nuclear factor-κB (NF-κB) in T cells, and acts synergistically with IL-12. It is markedly upregulated in CD.[38] There are several mechanisms that induce macrophages to the production of IL-12 and IL-18. NOD2 and Toll-like receptor must be important for bacterial products to induce IL-12 production. Further, IL-12 production is accelerated by osteopontin derived from intestinal plasma cells in CD.[39] The IL-12 p40 chain can form a heterodimer with p19 protein to form a recently described cytokine, IL-23.[40] In an IL-12p40 transgenic mouse model, constitutive p40 promoter activity is seen in the terminal ileum with high expression of IL-23 p19/p40 proteins in dendritic cells.[41] There are no publications on IL-23 in CD. L-21 is another newly described T-cell cytokine, with homology to IL-3, IL-4, and IL-15, which enhances Th1 signaling and IFN-γ production. It is increased in CD in comparison with UC and controls.[42] IL-17 is a cytokine with strong proinflammatory activity.[43,44] T-cell and macrophage production of IL-17 is significantly increased in both CD and UC but not in infective or ischemic colitis.[45]

The immunological basis for ulcerative colitis (UC) is much less clearly understood. Despite the evidence of a role for Th1 in CD, support for a Th2 pathogenesis in UC is much weaker. The presence of autoantibodies such as anticolon antibody, antimucin antibody, or antitropomyosin antibody is suggestive of a Th2 pathogenesis. Although there are several conflicting immunological findings, several immunological abnormalities exist and may induce colonic inflammation (Fig. 6). Mucosal T-cell production of IFN-γ is no higher than in controls, and although isolated T cells from UC patients make considerably more IL-5 than CD or control subjects, IL-4 production is reduced.[46] In mouse colitis induced by intracolonic injection of oxazolone, another Th2 cytokine, IL-13, produced by natural killer (NK) T cells, seems to be important.[47] Nonclassical NK T cells isolated from UC mucosa also produce markedly increased levels of IL-13 and are cytotoxic to epithelial cell targets.[48] IL-13 also increases epithelial permeability.[49]

STAT3 is involved in a wide variety of sometimes opposing signaling pathways. It is the major signaling molecule for the IL-6 family of cytokines but is also activated by IL-10, granulocyte colony-stimulating factor (G-CSF), and hepatocyte growth factor.[50] STAT3, activated phospho-STAT3, and the endogenous inhibitor of STAT3 signaling, SOCS3, are markedly increased in IBD patients compared with controls.[51] Myeloid cell-specific STAT3 deletion in a mouse model makes neutrophils and macrophages unresponsive to IL-10, and produces a slow-onset chronic Th1-mediated colitis, similar to that seen in IL-10 knockout mice.[52] IFN-γ-induced somatic inactivation of STAT3 in myeloid cells also triggers an aggressive and fatal colitis.[53] Deletion of STAT3 in the bone marrow during hematopoiesis leads to the development of a rapidly fatal, CD-like enteropathy.[54]

Intestinal macrophages in normal mice showed a bone marrow-derived macrophage phenotype, and thus act as antiinflammatory macrophages, producing a high amount of IL-10 in response to enteric bacteria. By using an IL-10 knockout mouse model, intestinal macrophages in colitis showed an inflammatory phenotype in response to enteric bacteria, and whole bacteria could induce IL-12 from tissue macrophages. Further, IL-10 supplementation could attenuate abnormal IL-12 production from macrophages, and this abnormal differentiation of macrophages is also found in some CD patients.[55]

The IL-6 cytokine family signals through the gp130-like receptor, activating both STAT3 and SHP-2/ras/Erk pathways. IL-6 when complexed with soluble IL-6 receptor can bind to cells lacking the IL-6R (trans-signaling). IL-6 trans-signaling is elevated in patients with IBD and enhances T-cell resistance to apoptosis. A neutralizing antibody to IL-6R induces T-cell apoptosis and prevents trinitrobenzene sulfonic acid (TNBS) colitis, and anti-IL-6 receptor antibody shows some promise for the treatment of CD.[56] The spontaneous IBD that occurs in mice with targeted disruption of STAT3 in immune cells is probably caused by a failure of IL-10 downregulation. At the same time, in models of immune-mediated gut inflammation in normal mice, IL-6 is overexpressed in the mucosa (as it is in IBD), and signaling through gp130 helps prevent mucosal T-cell apoptosis to drive inflammation.

Epithelial repair

The chronic inflammatory process leads to the disruption of the epithelial barrier and formation of epithelial ulceration. Resolution of inflammatory activity is associated with repair processes that facilitate tissue remodeling, which restores normal intestinal architecture.

Repair processes in UC patients are often effective in restoring a normal mucosal architecture, but stricture formation associated with excess fibrosis frequently occurs in CD patients.[57] Mesenchymal cells derived from bone marrow stem cells play a crucial role in the process of intestinal repair and fibrosis.[58,59]

Bone marrow (BM)-derived cells substantially repopulate the epithelia of the human gastrointestinal tract during regeneration.[60,61] BM-derived epithelial cells reside as progenitor cells residing in the crypt and also as terminally differentiated epithelial cells. Moreover, the proliferation and the differentiation of BM-derived cells toward secretory lineage epithelial cells are accelerated when epithelial regeneration is required, thereby contributing to the epithelial regeneration following severe inflammation of the human gastrointestinal tract.[62] In IBD patients, this epithelial repair process may be disturbed.

Conclusion

Important new insights have been gained recently into the pathophysiology of IBD, particularly in regard to CD. There is strong evidence for Th1-mediated response in the pathogenesis. Microbes or food antigens may directly stimulate macrophages and dendritic cells to produce Th1 cytokines and to activate T cells. In contrast, invasion of intestinal antigens or activation of immune cells is not easily induced under normal conditions. In IBD, this vicious circle is thought to accelerate the intestinal inflammation. Thus, more precise analyses regarding some genetic or environmental factors, immunological abnormalities, and epithelial repair disorder appear to be converging to explain the pathophysiology of IBD.

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