

とになると考えられている⁶⁾⁷⁾。

診 断

1. 患者基本情報

好酸球性食道炎患者は男性が多く、欧米の報告では76%が男性であると報告されている⁷⁾。日本の調査でも80%が男性であった。好発年齢は日本では26~79歳で平均51歳と中年であるが、欧米からの報告では平均38歳とやや若い。また成人例の23%に家族歴があったと報告されている。

日本人患者の22%の例で喘息の合併を認め、44%の例では何らかのアレルギー疾患を合併していた。欧米においても半数の患者でアレルギー疾患の合併があると報告されている。

2. 主訴

胸やけ、呑酸、嘔吐、嚥下困難、食物のつかえ、腹痛などが主訴となる⁸⁾⁹⁾。症状から胃食道逆流症 (gastroesophageal reflux disease; GERD)、特に非びらん性胃食道逆流症 (non-erosive reflux disease; NERD) と診断され、PPI (proton pump inhibitor) 抵抗性 GERD の中に好酸球性食道炎が混入しうることが報告されている¹⁰⁾。症状は年齢とともに変化し幼児期では発育障害や逆流症状、学童期では腹痛や嘔吐、成人期では嚥下障害や食べ物のつかえが主訴となりやすい。成人期では慢性的に持続してきた炎症のために食道の粘膜下の線維化が起り、食道が狭窄するので嚥下障害が起りやすいと考えられている。

日本で行った成人例を対象とした消化器内科での調査では、60%の好酸球性食道炎例が何らかの嚥下時不快感を訴えており、全36例のうち嚥下障害を16例が、つかえ感を7例が、胸痛を5例が、胸やけを3例が、心窩部痛を3例が、食欲不振を2例が、咽頭部不快感を2例が訴え(重複あり)、2例は無症状であった。

3. 血液検査

末梢血中の好酸球数の増加の有無は、欧米の報告では頻度はまちまちで報告によって10~100%のばらつきがある。日本での調査では30%の例に末梢血好酸球数の増加が認められている。日本人患者で最も多い例は8,774/ μ l、最も少ない例は162/ μ lであった。CRP (C-reactive protein) は、0.2

以下の正常値を示す例から2.9 mg/dl と高値を示す例まで分散していたが、異常高値を示す例は36例中4例のみであり、上昇するとしてもわずかであると考えられる。血中のIgEを測定すると70%の例でIgEの増加が証明され、RAST (radioallergosorbent) では様々な食物抗原や真菌に対してアレルギーの存在を証明することができる。

血漿中の eotaxin3 や IL-15 は、健常者と比較すれば高値を示す例もあるが、個人間でのばらつきが大きく、診断に用いるには現時点までの成績では感度や特異度が十分高くはない。

4. X線検査

硫酸バリウムを用いた食道のX線透視検査を行うと、食道壁に多発性の狭窄を認めることがある。狭窄は幅数 mm の輪状の狭窄がいくつも連続して形成されたものであり、慢性的な食道粘膜の炎症に起因する食道粘膜下層の線維化が原因であると考えられている。

胸部のCT検査を行えば、食道壁の著明な肥厚を64%の例に認める。食道壁に肥厚が存在する例で肥厚の存在部位を超音波内視鏡検査で観察すると、粘膜下層が主な病変部位であることがわかる。

5. 内視鏡検査

内視鏡検査を行えば、様々な異常を発見することができる。Müllerら⁹⁾は117例の好酸球性食道炎例の内視鏡像の検討を行い、white stippled-like exudates を25.6%の例に、linear fissures を25.6%の例に、reddening を25.6%の例に、rings を18.8%の例に、focal strictures を16.2%の例に、wrinkled pattern を14.5%の例に、cobble stone-like pattern を13.7%の例に、granulation を13.7%の例に、undulations を10.3%の例に、reduced vasculature を6.8%の例に、long segment stricture を4.3%の例に、scars を1.7%の例に認めると報告している。内視鏡検査を行っても24.8%の例では食道に異常は発見されていない。

日本における厚生労働省研究班による調査では調査された36例のうち35例において内視鏡検査の結果が記載されており、このうち16例(46%)で内視鏡検査で異常が発見されていない。Müllerら⁹⁾の報告と同様に日本人においても、好酸球食

道炎のかなりの例で内視鏡検査を行っても異常を発見できないことがわかる。

異常が存在する例の中で最も高頻度に発見されるのは、日本人においても white stippled-like exudates で、9例(26%)に発見されている。これは食道粘膜の表層に付着しているように見えるカンジダの白斑を小さくしたような多発する白斑である。白斑が形成される原因は必ずしも十分に明らかとなっているわけではないが、生検をして白斑部を組織学的に検索すると、扁平上皮の浅層部に好酸球が集簇した、eosinophile microabscess が発見されるとする報告がみられる¹¹⁾。このため、多発する白斑は上皮層中に多数の好酸球の浸潤があることを内視鏡検査で直接に示すサインである可能性があると考えられる。

次いで6例(17%)に rings (輪状狭窄) が、4例(11%)に linear fissures (縦走溝) が同定されている。rings は、食道の内腔の半周～全周性の数 mm の短い長さの狭窄を多数有する状態を示している。ちょうど気管の内を気管支鏡検査で観察したときにみられる気管軟骨の存在部位がやや狭くなっている様子と類似しており trachealization と呼ばれることもある。この rings ができる原因は、粘膜の慢性的な炎症のために粘膜下層の肥厚と癒痕収縮が起こり短い範囲の狭窄が起こったことに加えて、炎症に起因する食道輪走筋の spasms も関与していると推定されている。

linear fissures の原因については、検討がほとんどなされていない。好酸球性食道炎同様に粘膜上皮下に線維の増生がみられる collagenous colitis でも縦走する fissures が形成されることがあるが、好酸球性食道炎の linear fissures が同じ機序で起こっている可能性も推定でき、今後の検討が待たれる。白斑、発赤、輪状狭窄、縦走溝などの内視鏡所見以外にも、好酸球性食道炎例の粘膜を内視鏡下に生検すると粘膜上皮が大きく剥がれるように採取される現象は collagenous colitis でも報告されており、やはり粘膜上皮下の膠原線維の増生が示す特徴である可能性もある。

内視鏡検査を用いた診断で重要なことは、欧米からの報告では約 25% で、また日本での集計でも 46% の好酸球性食道炎例で内視鏡検査では異

常が見つからないことである。今後、より多数例を集積して詳細な観察を行う努力と、NBI (narrow band imaging), FICE (flexible spectral imaging color enhancement), 色素内視鏡などのより詳細な観察を可能とする手技を用いることで、好酸球性食道炎の内視鏡診断の感度と特異度を改善していくことが可能となると期待される。

Fig. 1~4^{4) 5) 12)} に筆者らの施設および関連の施設で経験した 4 例の好酸球性食道炎の内視鏡写真を示す。

6. 生検診断

好酸球性食道炎では 1/4~1/2 の例で内視鏡検査を行っても異常が発見できない。そこで内視鏡検査で異常がなくても食道に起因すると考えられる胸やけ、呑酸、胸痛、嚥下障害、つかえ感などの症状がある場合には、内視鏡下生検を行うことが重要となる¹³⁾。生検を行う食道内の部位については、白斑などの病変がみられた場合には、病変部を生検することが有用であろうと考えられる。病変が発見できなかった場合に、食道内のどの部位から生検を行えばよいかについて十分な検討はない。

好酸球が食道上皮内へ浸潤するためには CD4 陽性の T リンパ球が産生する IL-13 が上皮細胞に作用して産生される eotaxin3 が重要な役割を有している。培養した扁平上皮細胞に IL-13 を添加すると扁平上皮細胞は eotaxin3 を産生するが、このときに培養条件を pH 7.4 の中性から pH 4 の酸性状態とすると、同じ濃度の IL-13 による刺激でも産生される eotaxin3 の量が倍増することが報告されている¹⁴⁾。そこで、生理的な範囲 (%time pH < 4.0, すなわち食道粘膜表層が pH 4 以下の酸性条件に曝されている時間が、全計測時間の 4% 以下) でも胃酸の逆流に曝されやすい下部食道粘膜は上部食道粘膜に比較して好酸球の浸潤が起こりやすいことが推定される。実際に、好酸球性食道炎例の上、中、下部食道の上皮内好酸球の数を比較した報告では、上部食道では 1~157 (平均 63.3) 個/高倍率視野、中部食道では 3~182 (平均 62.1) 個/高倍率視野、下部食道では 18~244 (平均 91.5) 個/高倍率視野の好酸球が存在するとされており、下部食道のほうが好酸球が多い傾向

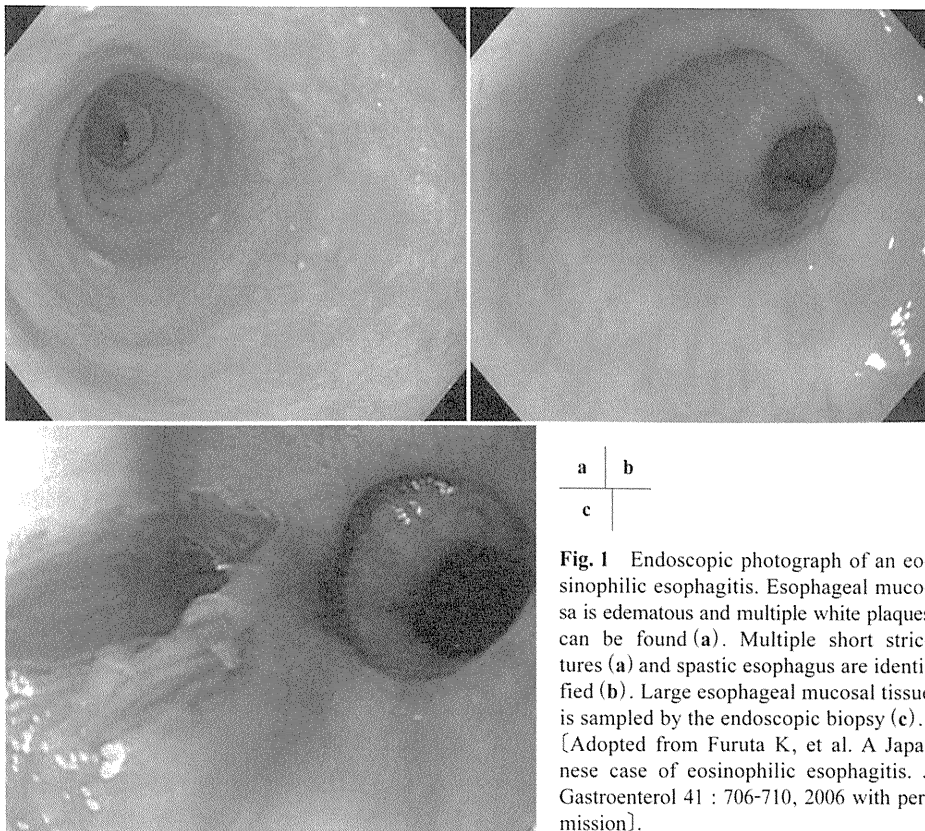


Fig. 1 Endoscopic photograph of an eosinophilic esophagitis. Esophageal mucosa is edematous and multiple white plaques can be found (a). Multiple short strictures (a) and spastic esophagus are identified (b). Large esophageal mucosal tissue is sampled by the endoscopic biopsy (c). [Adopted from Furuta K, et al. A Japanese case of eosinophilic esophagitis. J Gastroenterol 41 : 706-710, 2006 with permission].

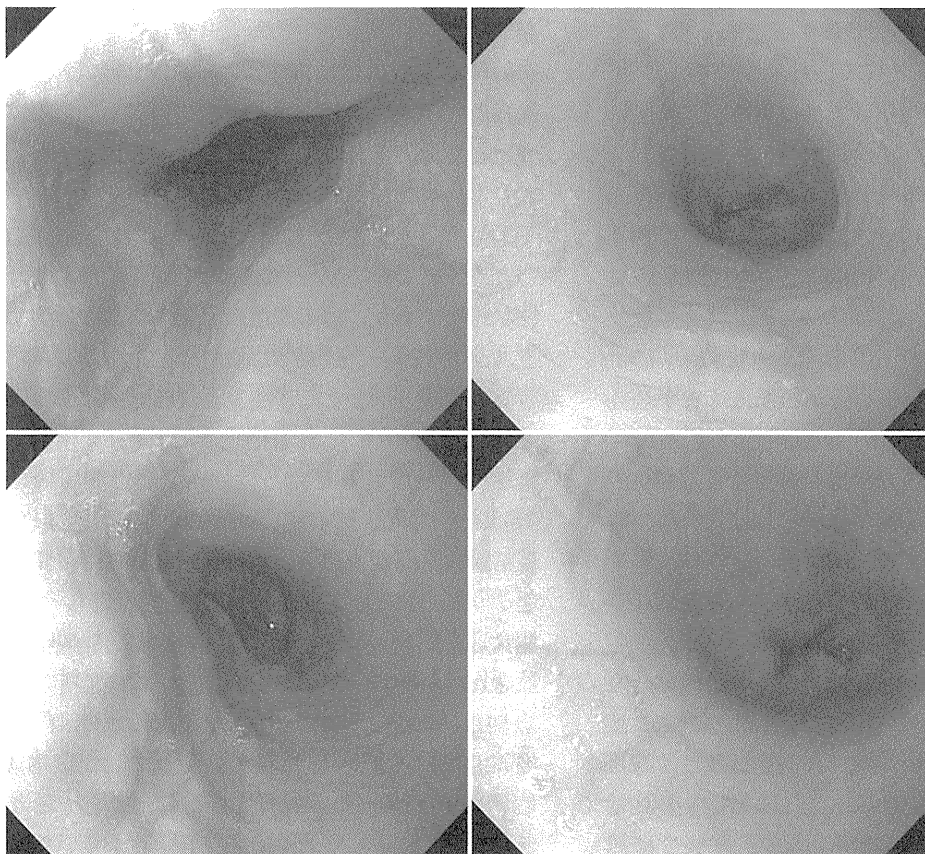


Fig. 2 Endoscopic photograph of an eosinophilic esophagitis. Multiple linear fissures are found. With smaller air insufflations, linear fissures are easily identified. Adopted from reference 4 with permission. [Adopted from 木下芳一, 他. 好酸球性食道炎の診断と治療. Gastroenterol Endosc 53 : 3-15, 2011 with permission].

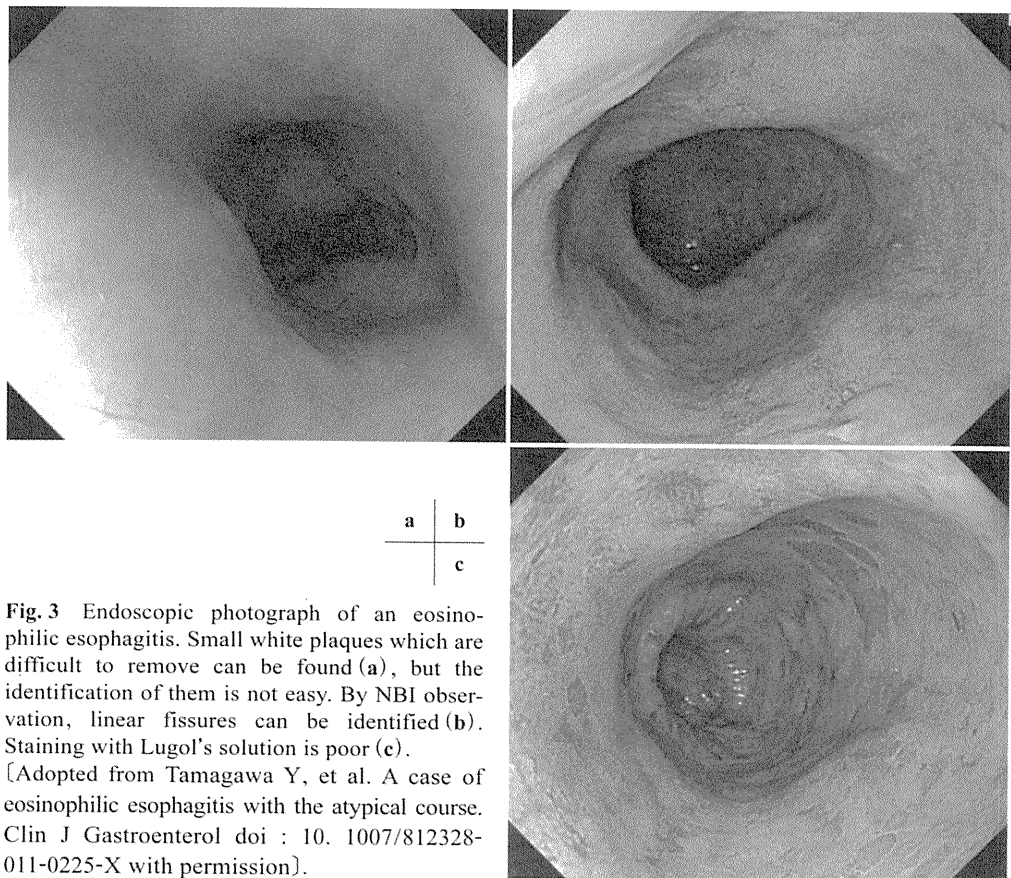


Fig. 3 Endoscopic photograph of an eosinophilic esophagitis. Small white plaques which are difficult to remove can be found (a), but the identification of them is not easy. By NBI observation, linear fissures can be identified (b). Staining with Lugol's solution is poor (c). [Adopted from Tamagawa Y, et al. A case of eosinophilic esophagitis with the atypical course. Clin J Gastroenterol doi : 10. 1007/812328-011-0225-X with permission].

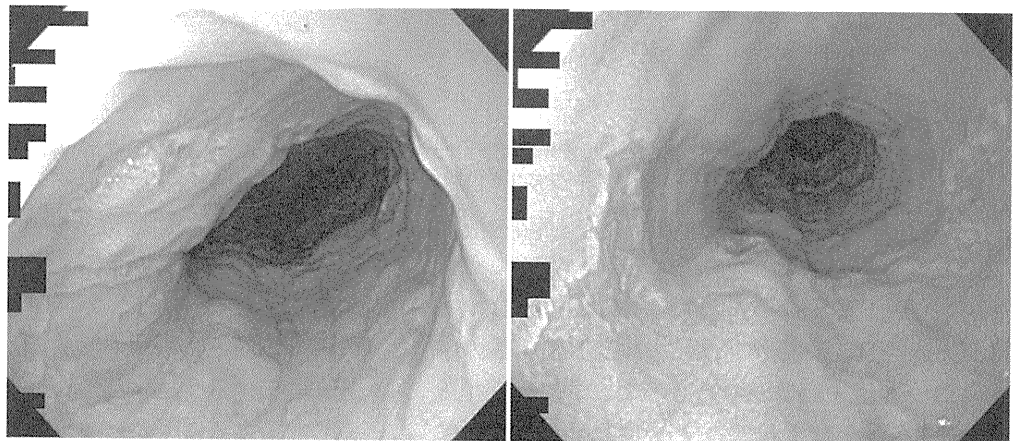


Fig. 4 Endoscopic photograph of an eosinophilic esophagitis. Mucosal undulation, edema, linear fissures can be found.

がみられる⁹⁾¹⁵⁾。また、好酸球性食道炎例の食道上皮内好酸球数は生検部位によって不均一であり、生検を1個行うだけでは、診断の感度は55%で、感度を100%にするには5個の生検が必要であるとされている。そこで、好酸球性食道炎の可能性を考えて生検を行う場合には、病変が存在する場合には病変部を、病変が同定できない場

合には下部食道を含めて、複数の生検組織を採取することが必要であろうと考えられる。

好酸球性食道炎と診断を確定するための上皮内好酸球の数については、必ずしもコンセンサスが得られているわけではない。20個/高倍率視野を確定診断に必要な食道上皮内好酸球とする報告が多いが、15~30個/高倍率視野とする論文もあ

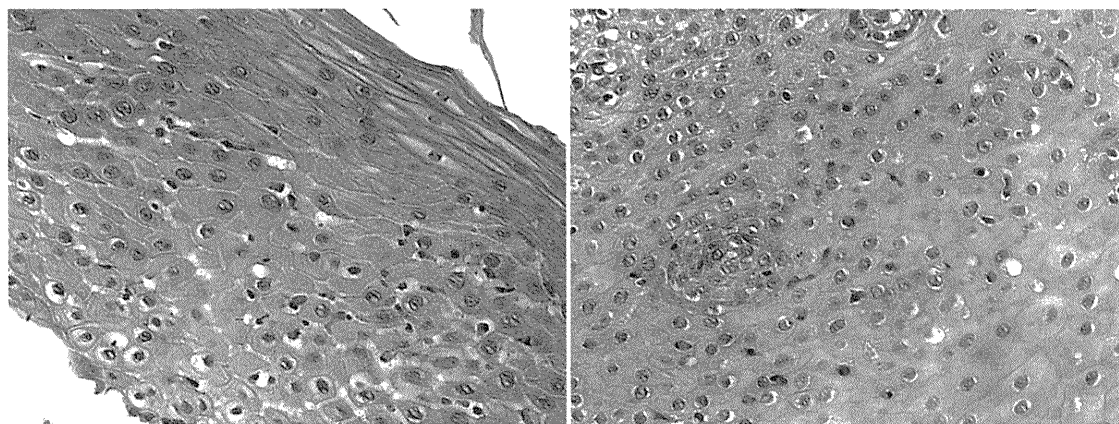


Fig. 5 Histopathological photograph of a biopsy specimen from an eosinophilic esophagitis. Many eosinophilic leukocytes can be found in the esophageal epithelium (HE, $\times 400$).
 [Adopted from Furuta K, et al. A Japanese case of eosinophilic esophagitis. *J Gastroenterol* 41 : 706-710, 2006 with permission].

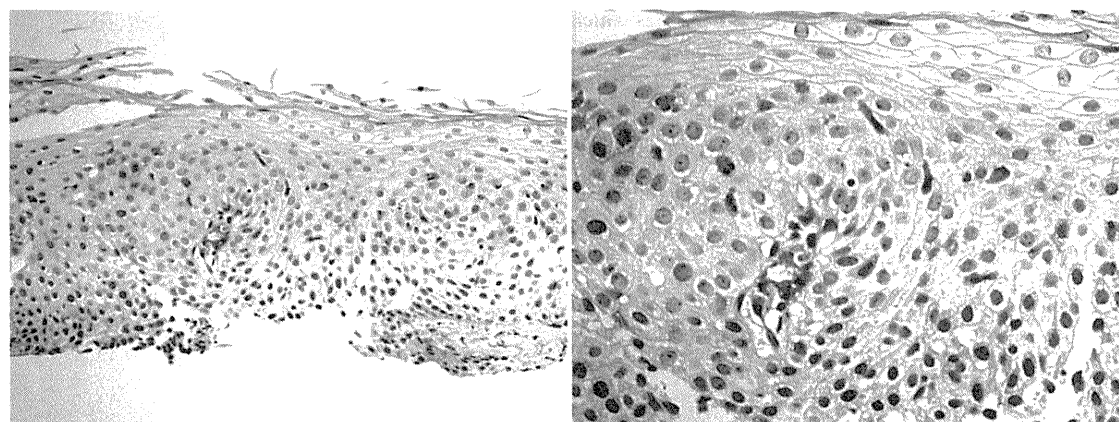


Fig. 6 Histopathological photograph of a biopsy specimen from an eosinophilic esophagitis. Many intraepithelial eosinophilic leukocytes and hyperplasia of basal cell layer are found (HE, a : $\times 100$, b : $\times 400$).
 [Adopted from 木下芳一, 他. 好酸球性食道炎の診断と治療. *Gastroenterol Endosc* 53 : 3-15, 2011 with permission].

る。また、上皮内好酸球は、複数の生検組織の中で1つでも、また1つの組織の中で1視野だけでも15~30個を超えれば好酸球性食道炎と診断すべきであるとされている。Fig. 5, 6に筆者らの施設で経験した2例の好酸球性食道炎の組織像を示す。

好酸球の食道上皮内浸潤はGERD例においても観察されることが知られている。ただし、GERDに起因する上皮内好酸球浸潤は5個/高倍率視野を超えることはほとんどなく、15~30個/高倍率視野の好酸球浸潤を有する好酸球性食道炎とは大きな差がみられる¹⁶⁾。さらに、GERDでは好酸球の浸潤は上皮層の粘膜下層に近い深い部分を中心

にみられるが、好酸球性食道炎ではむしろ浅い部分にみられ、好酸球のmicroabscessも表層部にみられることが多い。さらに、GERDとは異なって好酸球性食道炎では好酸球とともにマスト細胞やリンパ球の浸潤がみられることも特徴の一つである。

7. 生理学的検査

原則として食道pHモニタリング検査で、異常な胃酸の食道内逆流はみられないことが好酸球性食道炎と診断するための条件となっている。ただし、GERDはありふれた疾患であるため、GERDと好酸球性食道炎がたまたま合併することはまれではない。さらに、胃酸の食道内逆流はIL-13刺

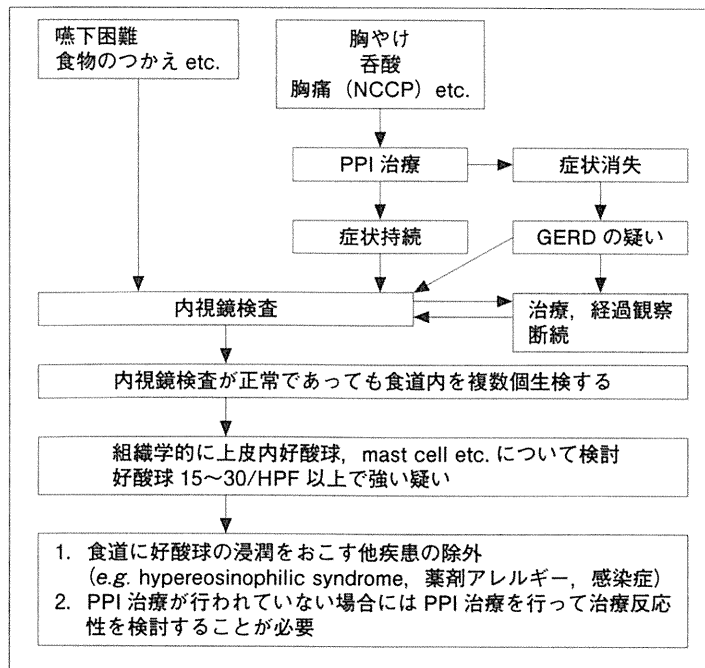


Fig. 7 好酸球性食道炎の診断プロセス.

激による上皮細胞での eotaxin3 の産生を増加させることにより好酸球性食道炎を悪化させる可能性もある。実際、好酸球性食道炎例に食道内 pH モニタリング検査を行うと 20% 程度の例で異常酸逆流があるとされている。

食道の運動能を調べるために食道内圧モニタリング検査を行えば、好酸球性食道炎例の約半数で異常が見つかる。約 40% の例では食道体部、特に下部食道の蠕動運動の非特異的な異常 (収縮力の低下, 亢進, 同期性収縮など) がみられ、約 10% の例では下部食道括約筋部の収縮力の低下がみられると報告されている。これらの異常は好酸球性食道炎をステロイドなどで治療することで自然と正常化するため、慢性の炎症に伴った二次的な異常であろうと考えられている。

8. 診断のプロセス

好酸球性食道炎の診断のプロセスを Fig. 7 に示す。食道由来であると疑われる症状を主訴とする患者を診た場合には GERD 例が多いため、まず PPI を用いて empirical therapy を行い GERD 例を除くことが現実的である。PPI 治療に抵抗する場合には内視鏡検査を行い、白斑、輪状狭窄、縦走溝などの異常の発見に努める。内視鏡検査で異

常が発見できなくても、下部食道を含む複数部位から生検診断を行う。上皮内好酸球が 15~30 個/高倍率視野以上みられた場合には食道に好酸球の浸潤を引き起こす、好酸球性胃腸炎, hyper-eosinophilic syndrome, 膠原病, 薬物アレルギーなどの可能性を検討したうえで、他に原因がなければ好酸球性食道炎と確定診断を行うことになる。

おわりに

好酸球性食道炎の主に内視鏡診断について解説したが、好酸球性食道炎の内視鏡診断の感度・特異度は現状では十分に高いものではない。症状や、家族歴、既往歴、また合併するアレルギー疾患の有無に関する十分な病歴の聴取、詳細な内視鏡観察、内視鏡検査で異常が全くみられなくても下部食道を含む食道内からの複数の生検組織の採取を行う慎重さが確定診断への道であることを理解しておくことが重要である。

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Summary

Endoscopic Diagnosis of Esophagitis — Eosinophilic Esophagitis

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Eosinophilic esophagitis is a chronic allergic disease aggravated by inhaled fungal antigens and food antigens. Patients with this disease report various esophagus related symptoms such as dysplasia and heartburn. Endoscopic examination frequently detects esophageal mucosal white stippled-like exudates, linear fissures, reddening, rings, and other mucosal changes. In one fourth of the investigated cases, however, no abnormality can be found even by endoscopy. Therefore, when patients report chronic symptoms possibly related to the esophageal diseases, endoscopic biopsies and histo-pathological examination of the esophageal mucosa should be seriously considered even in the absence of endoscopically-identified abnormalities.

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好酸球性食道炎 —注目の疾患—

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要 旨

- ・好酸球性食道炎 (eosinophilic esophagitis ; EE) は食道粘膜上皮内への好酸球浸潤を主とする炎症性(アレルギー性)疾患である。
- ・従来は小児科領域で多く報告されていたが、近年成人においてもつかえ感や食べ物のつまりを特徴とする疾患として認識されつつある。
- ・アトピー性皮膚炎、気管支喘息、食物アレルギーなどの疾患を合併しているケースが半数にみられる。
- ・厚生労働省の診断指針案では、嚥下障害やつかえ感などの症状を有し、食道粘膜生検で上皮内に 20/HPF 以上の好酸球が存在していることを必須項目としている。
- ・日本での実態調査では中年男性に多く、嚥下障害が主訴になりやすく、白斑、縦走溝などの特徴的な内視鏡像を認め、食道壁の肥厚が著明である。
- ・本疾患には IgE 介在型のメカニズムが発症に関与していると考えられている。
- ・厚生労働省の治療指針案としてはプレドニゾロンの内服、または吸入用フルチカゾンの投与をあげているが、投与量、減量スピード、中止の時期、再発、再燃時の対応については一定の見解は示されていない。
- ・ロイコトリエン D₄受容体拮抗薬、mepolizumab による抗 IL-5 療法、アザチオプリン、食事療法、拡張術などの治療法もあるが、現時点では第一選択とは考えられていない。
- ・アレルギー歴の聴取や原因不明のつかえ感、胸やけ、胸痛などの症状があれば、本疾患を疑って積極的な食道生検が必要である。

はじめに

好酸球性食道炎 (eosinophilic esophagitis ; EE) は食道粘膜上皮内への好酸球浸潤を主とする炎症性(アレルギー性)疾患であるが、1978 年 Landres らによって初めて報告された¹⁾。従来は小児科領域で多く報告されていたが、近年成人においてもつかえ感や食べ物のつまりを特徴とする疾患として認識されつつある。また、アトピー性皮膚炎、

気管支喘息、食物アレルギーなどの疾患を合併しているケースが約半数にみられ、5~58%の患者には好酸球増加を伴っている¹⁾。本疾患の有病率の増加には多因子が関与していると考えられるが、消化器病医や病理医の認識の高まり、アレルギー疾患の増加などもその一部と考えられる。

頻 度

EE は、ここ 5 年間で劇的に増加しつつあり、

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欧米では 30~40/100000 人程度の発症といわれており、Crohn 病のそれと同等である²⁾。また、スイスで最近行われた研究では、成人における EE 有病率が 16 年間で 2/100000 人から 27/100000 人へ増加したと報告されている³⁾。本疾患は若い男性に起こる傾向があるとされており、13 の研究において、323 人の成人患者のうち 76% が男性で、平均年齢が 38 歳(14~89 歳)であった²⁾。本邦での実態調査では 36 症例中 29 人は男性、女性は 7 人であった⁴⁾。平均年齢は 51 歳で海外のレビューと比べると若干年齢層は高い傾向にある。また、16 の研究において 754 人の小児の患者のうち 66% が男児、平均年齢が 8.6 歳(0.5~21.1 歳)だった。家族歴が認められている例もあり、381 人の小児の EE 患者のうち 5% の兄弟が、7% の親が EE であったという報告もある²⁾。

病態生理

本疾患には IgE 介在型のメカニズムが発症に関与していると考えられている。EE における好酸球関連サイトカインとケモカインは IL-5 とエオタキシンであり、IL-5 は好酸球の産生、活性化、生存に関与しているが、さらに好酸球を食道に遊走する役割も持っている。一方、エオタキシンは好酸球の胃腸への走化性に大きな役割を果たしている¹⁾。エオタキシン 3 は好酸球のケモタキシンに関与するサイトカインであるが、本疾患の食道粘膜ではエオタキシン 3 の発現上昇が報告されており、小児の EE 患者で特に高く認められている。成人の EE 患者の食道組織内のエオタキシン 3 に対する免疫組織学染色では GERD (gastroesophageal reflux disease) 例に比べ、染色性が高いことが知られている²⁾。

診断基準

厚生労働省研究班が提案している診断基準⁴⁾では、① 症状(嚥下障害、つかえ感など)を有する、② 食道粘膜の生検で上皮内に 20/HPF 以上の好酸球が存在している(生検は食道内の数カ所を行うことが望ましい)、③ 内視鏡検査で食道内に白斑、

縦走溝、気管様狭窄を認める、④ CT スキャンまたは超音波内視鏡検査で食道壁の肥厚を認める、⑤ 末梢血中に好酸球増多を認める、⑥ 男性、⑦ プロトンポンプ阻害薬(PPI)は無効でグルココルチコイド製剤が有効という 7 項目のうち、① と ② は必須でこれら以外のほかの項目も満たせば可能性が高くなるとしている。

欧米の診断基準では、食道 pH モニタリングにおいて異常な食道内酸曝露を認めないという記載があるものや PPI を用いた治療を 2 カ月間行っても症状が軽快しないという項目が付け加えられている場合もあるが、その他の点では日本と欧米の診断基準はほぼ同様の内容である。

症 状

本邦の実態調査では、36 例中 23 例(64%)が嚥下障害やつかえ感、3 例(8%)が胸やけを有していたが、1 例では症状はなかった。海外レビューでは嚥下障害(93%)、食物のつまり(61.9%)、胸やけ(23.6%)と報告されており、嚥下障害や食物のつまりは日本人においては本疾患を疑う重要な症状であると推察される。また、EE 患者 117 例を後向きに評価した最近の研究でも、つかえ感をもっともよくみられる症状(全例の 70%)であったと報告されている³⁾。

一方、つかえ感を主訴とする例における EE の頻度に関するデータは現時点ではきわめて限られており、小規模集団を対象とした後向き研究では 92% (12/13 例)であったと報告されている。また、子供では幼児期は吐き気、嘔吐、消化不良、食物拒否などの症状が多く⁵⁾、成人の症状とは違いがみられる。

検査所見

EE の診断に内視鏡検査は不可欠であるが、本邦の実態調査では、縦走する溝状の所見(linear furrowing) (25%)、白斑(white exudates) (25%)、リング状(気管支輪様)所見(transient or fixed circular rings) (17%)、狭窄の所見(2.9%)であった。一方、海外ではリング状の内視鏡所見(49.2%)、

白斑(15.8%), 狭窄(39.7%), 狭小化した食道(5.4%), 食道粘膜の脆弱, 浮腫(59.3%), 異常所見なし(8.8%)と報告されている⁴⁾。小児では1/3が内視鏡的には正常であるとも報告されており注意が必要である。CT, EUS(endoscopic ultrasonography)による食道壁の肥厚の有無を確認することも必要で, 厚生労働省の研究班の実態調査では22例にCTまたはEUSが施行され, 14例に食道壁の肥厚が認められている。

末梢血での好酸球増多の頻度は, 5%以上であった症例は22例(76%), 500/ μ l以上を認めた症例は14例(48%)であった。海外レビューでは30.8%の患者で末梢血中の好酸球増多を認めると報告されている。また, 成人でのEEでは末梢血中の全IgEは60~69%で上昇しているとされている。EEの診断においてもっとも重要視されるのは, 食道上皮内の多数の好酸球浸潤であるが, GERD患者でも食道内に好酸球浸潤がみられるため, 本疾患の診断との鑑別が問題となることがある。ただし, 好酸球浸潤の程度はGERDでは少ないことで区別される(5~10/HPF以下)。GERDの患者数は多いため, 本疾患とのオーバーラップがみられることがあり注意が必要である。

もう一つのEEの組織学的特徴は好酸球性微小膿瘍で, 食道上皮に4ないしそれ以上の連続した好酸球の集合体と定義される。これは25~45%の患者にみられ¹⁾, この微小膿瘍は内視鏡で観察される白苔(白色滲出物)に一致していると考えられる。

治療

厚生労働省の研究班の報告では, EEの治療指針の案⁴⁾としてはプレドニゾロン20~40mg/日の内服, あるいは吸入用フルチカゾンの投与をあげているが, 投与量, 減量スピード, 中止の時期, 再発, 再燃時の対応については一定の見解は示されていない。

ステロイドを中止すると, 症状と食道内好酸球浸潤が3~6カ月以内に再発することがあり, 長期的な治療法に関してどのように行っていくべきかについては結論が得られていない。本邦の実態

調査でのステロイド療法については, 90%以上の例において少なくとも一時的な改善がみられている。海外レビューでは, コルチコステロイドの全身投与, 局所投与の有効性が示されている。コルチコステロイドの全身投与は症状を1週間以内に, 組織所見を4週間以内に改善することが報告されている。また, コルチコステロイドの吸入薬を用いた食道の局所ステロイド療法も即効性に症状軽減をもたらし有効率は95%であったと報告されている。

抗アレルギー薬の有効性については一定しないとされている。ロイコトリエンD₄受容体拮抗薬, mepolizumabによる抗IL-5療法, アザチオプリンなどを使用した報告があるが, ある程度の効果は認められるものの中止後の再発率も高く, 副作用などの問題からも第一選択の治療法とは現時点では考えられていない²⁾。これ以外の治療についても報告が散見されるが, 先述したようにGERDとのオーバーラップも多いことと, pHモニタリングでEE例の18%に異常がみられるという報告があることから, 本疾患の診断の前に, 診断的アルゴリズムの一つとしてPPIを使うことを推奨している報告もある²⁾。

投薬以外では食事療法が有効な例もみられる。アレルギーテストに基づいた食事制限(大豆, 卵, 麦, ピーナッツ, 海産物などを除去)をしたところ, 76%の患者で50%以上の好酸球減少が得られた報告もある²⁾。つかえ感や他の食道の症状が, ある特定の食物に曝露された後に起こることがあり, 食物摂取が食道のアレルギー反応の原因となり, 急性の粘膜浮腫をきたす結果であると推測されている。

食道狭窄を認める場合, しばしば拡張術が行われることがあるが, 3~8カ月で再発することが多いとされている。本疾患の食道粘膜は脆弱で, 「クレープペーパー」と形容されること⁶⁾があり, 粘膜裂創をきたしやすい。このため重大な合併症として穿孔が起こりやすいと考えられている。ただ, Jacobsらの報告では, 拡張術の穿孔率は671の拡張術に対し1例のみ(0.1%)であり, 特別に高い可能性もある⁷⁾。いずれにしてもEEによる炎症がコントロールできない場合, 反復して拡張が

必要となることが考えられるため、EE の病勢を制御するための治療を行うとともに、拡張術の施行にあたっては適応をよく検討する必要があると思われる。

症 例

ここに参考として本邦初の EE 症例を呈示する。本例は症例報告として、“Journal of Gastroenterology” に 2006 年に報告しており、詳しくは文献での原文を参照されたい。

症例は 69 歳、男性、4 週間続く腹痛と食欲不振で入院となった。他院で PPI 投与された経緯があるが、症状改善はなく、また食道由来の嚥下障害や胸痛などの症状は認めなかった。過去に上部内視鏡検査歴はなく、糖尿病、気管支喘息、高血圧などあり吸入ステロイド、テオフィリン、SU 薬、カルシウム拮抗薬を数年間内服していた。

検査所見では末梢血の好酸球の増加(2179/ μ l)、ハウスダストや真菌などに対する IgE の上昇を認めた。CT では食道の著明なびまん性壁肥厚を認め、バリウム透視では中～下部食道の狭小化を認めたが通過は良好であった。胃、十二指腸には異常はみられなかった。上部内視鏡検査では、食道粘膜は蒼白で、白苔付着(真菌感染に類似)、リング状所見を認め(図 1)、生検で大きな粘膜の裂創を形成した。病理組織学的には好酸球浸潤 20/HPF で、EUS では粘膜下層の著明な壁肥厚(図 2)を認め、通常より低エコーに描出された。24 時間 pH モニタリング検査では異常酸逆流は認めなかった。食道内圧検査では、高振幅の食道収縮(>150 mmHg)を伴う高頻度の食道の非蠕動性収縮を認めた。

以上の検査所見から EE と診断し、経口的なプレドニゾン 40 mg の内服から治療を開始した。2 週間後生検組織では好酸球浸潤は改善し、プレドニゾンを漸減したが、治療開始して 3、7 カ月後の EUS と食道造影では、食道狭窄と粘膜下層の肥厚に明らかな改善はなかった。

本症例では、喘息に対し数年前からフルチカゾン吸入をしていたが EE のコントロールは不良であった。過去にフルチカゾンを 1760 μ g/日を 6 週

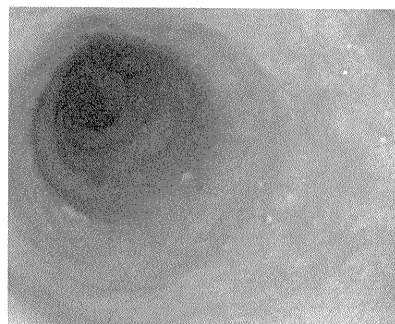


図 1 上部内視鏡所見¹⁾

(巻頭カラー参照)

食道粘膜は蒼白で、白苔付着、リング状所見を認める。

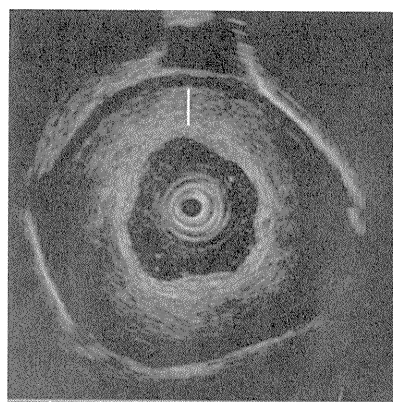


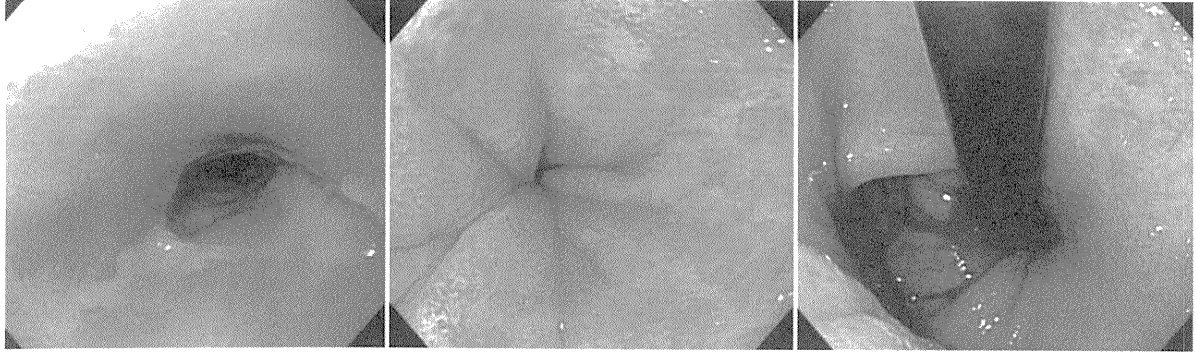
図 2 EUS 所見¹⁾

粘膜下層の著明な壁肥厚所見(白線)を認める。

間継続したケースで改善がみられた報告があるが⁸⁾、喘息に対するフルチカゾンの通常量は 200 μ g であるため、EE の治療効果に十分量ではなかったからかもしれない。

また、EE では特徴的な食道壁の肥厚を示すことがあるが、本症例でも EUS で食道の粘膜下層に著明な壁肥厚がみられている。ステロイド治療後でも通常の粘膜下層より低エコーで描出された理由については、浮腫性的変化、炎症、線維化などが考えられたが、好酸球の減少後でも壁肥厚が残存していたため、長期間の炎症が粘膜下層に線維化をもたらしたと考えられた。

また、EE の患者においては食後の非蠕動性収縮や食道の高振幅の収縮がみられることが報告されており、本症例でもこのような収縮異常が認め



|B|C

図4 中部食道癌—放射線単独治療 66 Gy 治療直後

食道癌は縮小し白苔は残存するも内視鏡が容易に通過した。

A：下部食道を中心に全周性にあたかも Angioectasia のごとく毛細血管の拡張と浮腫を伴い、易出血性の粘膜を認めた。

B：胃体上部も著明な易出血性の粘膜であった。

C：症状は急性期のみでも粘膜障害はその線量により比較的長期にわたることもある。

相見正史 他(pp906-910) 図1



図1 上部内視鏡所見¹⁾

食道粘膜は蒼白で、白苔付着、リング状所見を認める。

井上晴洋 他(pp911-914) 図2



図2 POEMの内視鏡写真

内輪筋の切開；三角ナイフで横に走る筋線維を捕捉して切離する。

られた。このため、異常な食道運動機能を有する患者を診断した時にも、EE も考慮するべきであると思われる。

おわりに

本疾患は比較的まれな疾患であるが、臨床医、病理医の認識が高まりつつあり、今後増加している可能性があると思われる。日常見逃されているケースも多々あることが予想され、アレルギー歴の聴取や原因不明のつかえ感、胸やけ、胸痛などの症状があれば積極的な食道生検が必要であり、まず「探す」ことから始めなければならない。本邦でも実態調査が進み、少しずつ病態が解明されつつあるが、今後さらなる症例の蓄積とそれに基づく標準的治療法の確立が望まれる。

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Increased Susceptibility to Autoimmune Gastritis in Thymic Stromal Lymphopoietin Receptor-Deficient Mice

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Thymic stromal lymphopoietin (TSLP), mainly produced by epithelial cells, activates a variety of cell types, including dendritic cells, mast cells, T cells, and B cells. It is involved in the pathogenesis of allergic inflammation in the lung, skin, and gastrointestinal tract. In addition, TSLP promotes Th2-type intestinal immunity against helminth infection and regulates Th1-type inflammation in a mouse model of colitis, suggesting that it plays crucial roles in intestinal immune homeostasis. Although autoimmune gastritis (AIG), mediated by inflammatory Th1 responses, develops in the gastric mucosa, it is not clear whether TSLP is involved in regulating these responses in AIG. The aim of this study was to examine the roles of TSLP in the development of AIG. Because BALB/c mice thymectomized 3 d after birth (NTx mice) develop AIG, we used this model to test the role of TSLP in the development of AIG. We found that in AIG-bearing mice, TSLP was expressed in the inflamed stomach and that the serum anti-parietal cell Ab levels in neonatal thymectomized TSLPR-deficient mice (NTx-TSLPR^{-/-} mice) were significantly elevated over those in NTx-TSLPR^{+/+} mice. In addition, NTx-TSLPR^{-/-} mice exhibited an earlier onset of AIG than that observed in NTx-TSLPR^{+/+} mice. The rapid development of AIG in NTx-TSLPR^{-/-} mice resulted in more aggressive CD4⁺ T cell infiltration and more severe loss of parietal and chief cells in the progression phase of AIG, accompanied by enhanced production of IL-12/23p40 and IFN- γ . Taken together, these data suggested that TSLP negatively regulates the development of AIG. *The Journal of Immunology*, 2012, 188: 190–197.

Autoimmune gastritis (AIG) is a typical organ-specific autoimmune disease. Patients with AIG often have complications, such as gastric cancer, gastric carcinoid tumors, or pernicious anemia accompanied by achlorhydria (1–3). The histological findings of AIG are characterized by a chronic mononuclear cell infiltration affecting only or predominantly the corpus mucosa and causing loss of parietal and chief cells from the gastric gland (1). AIG's serologic hallmark is the production of characteristic circulating autoantibodies, including Ab against

H⁺K⁺-ATPase, in the parietal cells of the stomach (4, 5). Mouse models of AIG share many pathological and clinical features with human AIG and help to clarify the mechanisms involved in its development (6).

BALB/c mice thymectomized 3 d after birth (NTx mice) are one of the mouse models of AIG. NTx mice possess disease-relevant CD4⁺CD25⁺ regulatory T cells (Tregs), but these Tregs cannot fully prevent autoimmune disease development (7–9). Adult NTx mice frequently develop AIG, showing lymphocytic infiltration with selective loss of parietal and chief cells from the gastric mucosa, as well as production of autoantibodies to parietal cells (10). In addition, AIG in NTx mice is characterized by a marked infiltration of CD4⁺ T cells, which produce large amounts of IFN- γ . The development of AIG in NTx mice is severely impaired in mice with depleted CD4⁺ T cells or in those receiving blocking Abs to IFN- γ (11–14). Therefore, the inflammatory Th1 responses induced by CD4⁺ T cells are critical for the development of this animal model of AIG.

Thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine, is mainly produced by epithelial cells and activates many hematopoietically derived cells, including dendritic cells (DCs), mast cells, T cells, and B cells (15, 16). TSLP is involved in the development of allergic inflammation in various organs, including the gastrointestinal tract (15–19). Interestingly, TSLP promotes Th2-type intestinal immunity against helminth infection and regulates Th1-type inflammation in mouse models of colitis (20, 21), suggesting that it plays crucial roles in intestinal immune homeostasis. Although AIG is also mediated by the inflammatory Th1 responses developed in the gastric mucosa, it is not clear whether TSLP is involved in regulating the development of AIG.

In the current study, to examine whether TSLP affects the development of AIG, we used BALB/c TSLP receptor (TSLPR)-deficient mice thymectomized 3 d after birth (NTx-TSLPR^{-/-} mice). We found that in AIG-bearing mice, TSLP was expressed

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Abbreviations used in this article: AIG, autoimmune gastritis; DC, dendritic cell; GLN, paragastric lymph node; MLN, mesenteric lymph node; NTx mice, BALB/c mice thymectomized 3 d after birth; NTx-TSLPR^{-/-} mice, thymic stromal lymphopoietin receptor-deficient BALB/c mice thymectomized 3 d after birth; PD-1, programmed cell death 1; Treg, regulatory T cell; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor.

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in the inflamed stomach. In comparison with NTx-TSLPR^{+/+} mice, NTx-TSLPR^{-/-} mice exhibited increased production of anti-parietal cell Abs, as well as early onset and enhanced severity of inflammation of the gastric mucosa. These data suggested that TSLP regulates the development of AIG.

Materials and Methods

Mice

BALB/c mice were purchased from Japan SLC (Shizuoka, Japan), and TSLPR-deficient mice on a BALB/c background were generated, as described previously (22, 23). In this study, we used TSLPR^{-/-} mice that had been backcrossed onto a BALB/c background for 12 generations. All of these mice were bred and housed under specific pathogen-free conditions. Thymectomy of the mice 3 d after birth was performed, as described previously (24, 25). All mouse protocols were approved by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University.

Histologic examination

After 24 h of starvation, mice were sacrificed, and the stomachs were immediately removed. The local pH in the corpus area of the stomach was measured with pH test paper (Advantec, Tokyo, Japan). Half of each stomach was fixed in neutral buffered formalin, embedded in paraffin wax, and cut into sections 4 μ m thick. These sections were stained with H&E. The other half of each stomach was frozen for immunohistochemistry. The degree of gastritis was determined according to a modification of a semi-quantitative scoring system, as described previously (26). Chronic inflammation, characterized by infiltration of mononuclear cells, was graded from 0 to 3, where 0 = no increase in inflammatory cells, 1 = slight infiltration of the lamina propria by lymphocytes, 2 = moderately dense infiltration, and 3 = very dense infiltration. Atrophic changes were graded from 0 to 3, according to the loss of specialized cells, chief cells, and parietal cells, (0 = no loss; 1 = mild loss of specialized cells, limited to half of the corpus glands; 2 = moderate loss of specialized cells, diffusing to more than half the corpus glands; and 3 = severe loss/almost complete loss of specialized cells throughout the gastric body). The degree of foveolar hyperplastic change of mucus neck cells of the corpus glands was scored on a scale of 0 to 3 (0 = no hyperplastic change; 1 = focal hyperplastic change of mucus neck cells of the corpus glands; 2 = moderate hyperplastic change of mucus neck cells, diffusing to the corpus glands with less than twice the height of a normal foveolar epithelial layer; and 3 = severe hyperplastic change of mucus neck cells, diffusing to the corpus glands with more than twice the height of a normal foveolar epithelial layer). Incidence of AIG was determined by greater than grade 2 chronic inflammation, characterized by moderately dense infiltration of mononuclear cells. These infiltrates were further confirmed to be CD4⁺ cells by immunohistological staining using FITC-conjugated anti-CD4 (eBioscience, San Diego, CA).

Immunohistological analysis

Fluorescence immunohistology was performed on frozen sections, as described previously (25). In brief, sections of 6 μ m were cut from tissue blocks of frozen mucosal samples onto glass slides. The sections were air dried for 30 min, fixed in acetone for 5 min, and blocked with PBS containing 1% BSA for 30 min. The sections were stained with FITC-conjugated anti-CD4 for 1 h or with biotinylated anti-TSLP (R&D Systems, Minneapolis, MN) for 1 h, followed by staining using Alexa Fluor 488-conjugated donkey anti-goat IgG (Invitrogen, Carlsbad, CA). After the final wash, the slides were mounted by Mowiol (Merck Chemicals, Darmstadt, Germany) and examined under a fluorescence microscope. Cell numbers of CD4⁺ T cells in the gastric mucosa were counted under high magnification. Briefly, 10 complete longitudinal profiles of gastric units were selected at random from the body of the stomach in each mouse, and the numbers of CD4⁺ cells/gastric unit were counted. Data were expressed as the average number of cells/section of gastric unit for each animal. For detection of autoantibodies for the gastric gland, stomachs were collected from wild-type BALB/c mice. Sections were stained with 100 \times diluted sera from the mice, followed by FITC-conjugated anti-mouse IgG (Southern Biotech, Birmingham, AL).

Real-time quantitative RT-PCR

Real-time quantitative RT-PCR was performed, as described previously (27). After the paragastric lymph node (GLN) was located and removed, gastric tissues were frozen in RNAlater (Qiagen, Hilden, Germany). Total

RNA was extracted using an RNeasy minikit (Qiagen), according to the manufacturer's instructions. Single-stranded cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen). Real-time quantitative RT-PCR was performed using SYBR Green I Master (Roche Applied Science, Basel, Switzerland). The real-time quantitative reactions were performed using a LightCycler 480 (Roche Applied Science), according to the manufacturer's instructions. Values are expressed as arbitrary units relative to GAPDH. The following primers were used: *GAPDH*: 5'-CAACTTTGT-CAAGCTCATTTCC-3' and 5'-GGTCCAGGGTTCTTACTCC-3'; *TSLP*: 5'-CAGCTTGTCTCCTGAAAATCG-3' and 5'-AAATGTTTTGTCCGGG-GAGTG-3'; *T-bet*: 5'-TCAACCAGCACCAGACAGAG-3' and 5'-AAACA-TCTGTAAATGGCTTGTG-3'; *GATA3*: 5'-TTATCAAGCCCAAGCGAAG-3' and 5'-TGGTGGTGGTCTGACAGTTC-3'; *ROR γ* : 5'-CCGCTGAGAG-GGCTTCAC-3' and 5'-TGCAGGAGTAGGCCACATTACA-3'; *IFN- γ* : 5'-GGATGCATTATGAGATTGC-3' and 5'-CCTTTTCCGCTTCTGAG-3'; *IL-4*: 5'-CGCCATGCACGGAGATG-3' and 5'-ACGAGCTCACTC-TCTGTGGTGT-3'; and *IL-17A*: 5'-TTTACTCCCTGGCGCAAAA-3' and 5'-CTTCCCTCCGATTGACAC-3'.

ELISA

Serum anti-parietal cell Ab levels were measured by ELISA, as described previously (26). Briefly, duplicate wells of microtiter plates (Nunc, Roskilde, Denmark) were incubated with 10 μ g/ml Ags, extracts prepared from the normal gastric mucosa, in PBS for 16 h at 4°C. The wells were blocked with PBS containing 5% nonfat dried milk and then incubated with serial dilutions of sera for 1 h. The wells were then incubated with HRP-labeled goat anti-mouse IgG (Serotec, Oxford, U.K.) diluted at a predetermined concentration for 1 h at room temperature. After rigorous washing, each well was reacted with substrate solution (R&D Systems) for 10 min. The reaction was terminated with 50 μ l 2 mol/l H₂SO₄, and absorbency at 490 nm was determined with a microplate reader.

Isolation of mononuclear cells

Single-cell suspensions from the tissues were prepared, as described previously (25, 28). In brief, mice were sacrificed, and the stomach, GLN, mesenteric lymph node (MLN), and spleen were immediately removed. After the removal of GLN, the stomach was opened, and stomach contents were removed by rinsing several times in PBS. The stomach tissue was repeatedly injected with a total of 10 ml PBS with 5% FBS using a 5-ml syringe attached to a 26-g syringe needle. Following the injections, the mucosa was gently massaged with the needle and was cut to help release any trapped cells. The cell suspension was filtered sequentially through 70- μ m nylon mesh. Collected cells were placed on a 40/75% discontinuous Percoll gradient (GE Healthcare, Little Chalfont, U.K.) and centrifuged at 20°C for 20 min.

Flow cytometry

Flow cytometric analysis was performed, as described previously (25, 27). Cells were stained with allophycocyanin-Cy7-conjugated anti-CD4 (BD Biosciences) and PE-conjugated anti-CD3 (eBioscience). Stained cells were analyzed with FACSCanto II (BD Biosciences). Data were analyzed using Cell Quest Pro (BD Biosciences). Dead cells were excluded based on side- and forward-scatter characteristics. The number of viable indicated cells was calculated as follows: (percentage of cells in the cell type) \times (number of viable cells).

Intracellular cytokine staining

Intracellular cytokine staining was performed, as described previously (25, 27). For T cell cytokine production, isolated cells were restimulated with 50 ng/ml PMA (Sigma, St. Louis, MO) + 2 μ g/ml ionomycin (Sigma) at a concentration of 1×10^6 cells/ml in RPMI Medium 1640 (Invitrogen), supplemented with 10% (v/v) heat-inactivated FCS (Sigma), penicillin G, streptomycin (both from Invitrogen), and 2-ME (Nacalai Tesque, Kyoto, Japan). After 2 h, brefeldin A (Sigma) was added at 10 μ g/ml. After 2 h, cells were collected and stained for cell surface molecules using allophycocyanin-Cy7-conjugated anti-CD4 and PE-conjugated anti-CD3. Cells were fixed and permeabilized using a Fix & Perm Cell Permeabilization Kit (Caltag Laboratories, An Der Grub, Austria) and stained with FITC-conjugated anti-IFN- γ (eBioscience). For DC cytokine production, isolated cells were cultured at a concentration of 1×10^6 cells/ml with brefeldin A. After 2 h, cells were collected and stained for cell surface molecules using allophycocyanin-conjugated anti-CD11b (eBioscience) and FITC-conjugated anti-CD11c (BD Biosciences). Cells were fixed, permeabilized, and stained with PE-conjugated anti-IL-12/23p40 (clone C17.8; eBioscience).

Statistical analysis

Statistical analysis was performed using the Student *t* test for paired or unpaired data to compare the values between two groups and using the Wilcoxon *t* test for nonparametric paired data. The incidence of AIG was compared with the Fisher exact test. The *p* values < 0.05 were considered significant.

Results

Inflamed stomach in AIG-bearing mice exhibits enhanced expression of TSLP

Increased TSLP expression in epithelial cells is induced through exposure to viral, bacterial, and parasitic pathogens, as well as to various cytokines (15, 16). First, we examined whether expression of TSLP was affected in the inflamed stomachs of mice with experimental AIG. We examined TSLP gene expression in AIG-bearing NTx mice, finding that these mice exhibited significantly elevated levels of mRNA expression of TSLP in the inflamed stomach compared with those in the normal stomach of BALB/c mice of the same age (Fig. 1A). In addition, to confirm this finding, frozen stomach sections from AIG-bearing NTx mice were stained with anti-TSLP Abs. Ig isotype-control Abs did not produce any positive staining. In contrast to immunostaining for

TSLP in normal gastric mucosa, we found obviously detectable anti-TSLP staining of epithelial cells in the inflamed gastric mucosa from AIG-bearing NTx mice (Fig. 1B).

TSLPR deficiency exaggerates autoantibody production in the mouse model of AIG

A previous study reported low-level TSLP gene expression throughout the small and large intestine, as well as that TSLP regulated a mouse model of colitis (20, 21). To test the possibility that TSLP is involved in the regulation of AIG, we performed thymectomy 3 d after birth in TSLPR^{-/-} or TSLPR^{+/+} mice in BALB/c background. We found that parietal cells of the stomach section in normal BALB/c mouse were stained with 100× diluted sera from 14-wk-old NTx-TSLPR^{-/-} and NTx-TSLPR^{+/+} mice but not normal BALB/c mice, indicating that both NTx-TSLPR^{-/-} and NTx-TSLPR^{+/+} mice developed AIG and that sera from these mice contained specific anti-parietal cell Abs (Fig. 2A). Using an ELISA

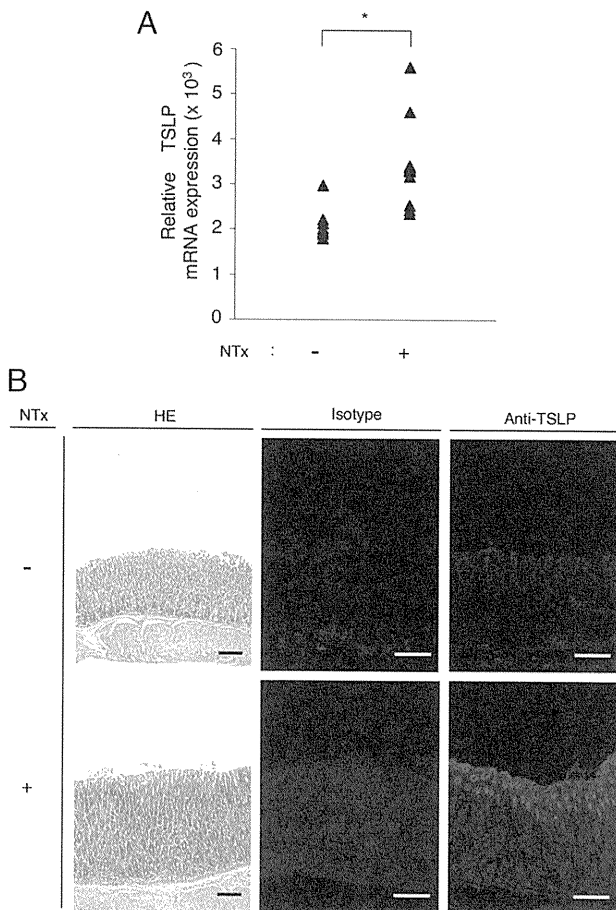


FIGURE 1. Inflamed stomach in AIG-bearing mice exhibits enhanced expression of TSLP. **A**, TSLP mRNA expression in inflamed gastric tissues from AIG-bearing NTx mice and normal controls from BALB/c mice at 12 wk of age. Expression levels of mRNA encoding TSLP were measured using real-time quantitative RT-PCR. Each symbol represents relative TSLP expression in an individual mouse. **p* < 0.05, Student *t* test for unpaired data. **B**, Immunohistological staining of the gastric mucosa. Inflamed gastric mucosa from AIG-bearing mice (lower panels) and normal mucosa from BALB/c mice (upper panels) were stained with H&E (HE), Ig isotype control Abs (Isotype), or anti-TSLP. Scale bars, 100 μ m.

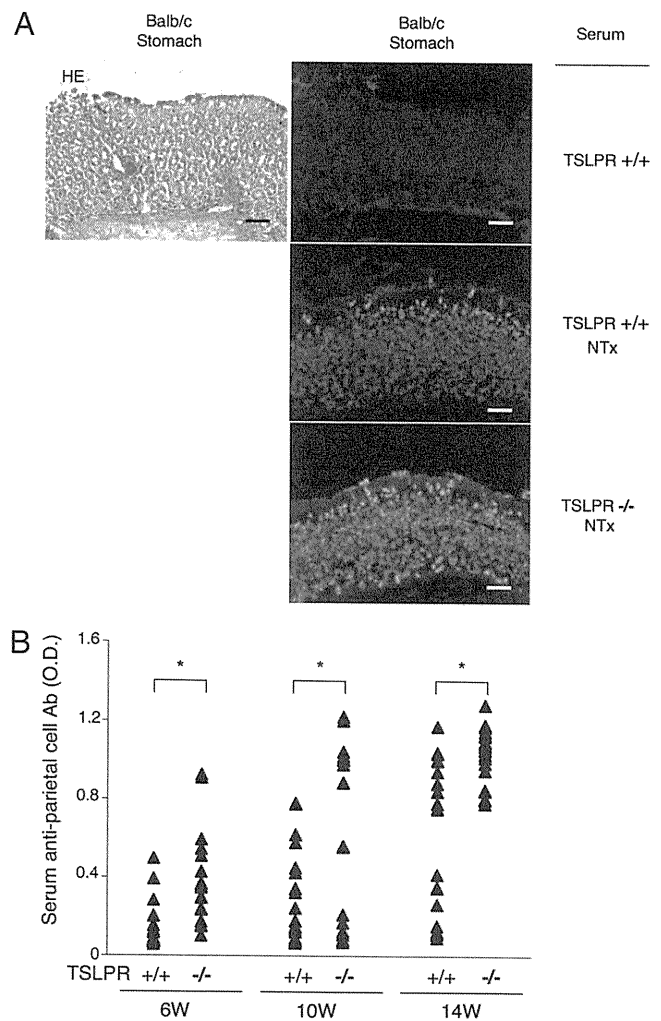


FIGURE 2. TSLPR deficiency exaggerates autoantibody production in the mouse model of AIG. TSLPR^{+/+} and TSLPR^{-/-} mice in BALB/c background were performed NTx. Sera were collected from indicated mice. **A**, Autoantibodies detected by fluorescence immunohistology. Gastric tissues of normal BALB/c mice at 12 wk of age were stained with H&E (left panel) or with 100× diluted sera from indicated 14-wk-old mice, followed by FITC-conjugated anti-mouse IgG (right panels). Scale bars, 100 μ m. **B**, Serum anti-parietal cell Ab levels were measured by ELISA. Each symbol represents OD in serum from individual NTx-TSLPR^{+/+} (*n* = 18) and NTx-TSLPR^{-/-} (*n* = 18) mice at 6, 10, and 14 wk. **p* < 0.05, Wilcoxon *t* test.

to examine the serum levels of anti-parietal cell Abs, we compared the levels of production of anti-parietal cell Abs. In 6-wk-old NTx mice, serum levels of anti-parietal cell Abs in NTx-TSLPR^{-/-} mice were significantly higher than those in NTx-TSLPR^{+/+} mice (Fig. 2B). In addition, although anti-parietal cell Ab titers gradually increased in NTx-TSLPR^{+/+} mice in an age-dependent manner, serum levels of anti-parietal cell Abs in NTx-TSLPR^{-/-} mice were significantly greater than those in NTx-TSLPR^{+/+} mice at 10 and 14 wk of age (Fig. 2B). These results suggested that TSLPR deficiency exaggerates autoimmunity in this mouse model of AIG.

TSLPR deficiency induces elevated histopathology of AIG in mice

Next, we examined whether TSLPR deficiency exacerbated gastric inflammation in our mouse model of AIG. Histological examination revealed that the gastric mucosa in 12-wk-old NTx-TSLPR^{+/+} mice had chronic gastritis with mononuclear cell infiltration, loss of parietal and chief cells, and hyperplasia of the foveolar mucus neck cells. Compared with the findings in NTx-TSLPR^{+/+} mice, NTx-TSLPR^{-/-} mice showed more severe mononuclear cell infiltration and complete loss of parietal and chief cells, accompanied by enhanced hyperplasia of the foveolar mucus neck cells (Fig. 3A). These findings were further confirmed by the gastric pH and gastritis scoring system that evaluates chronic inflammation, characterized by the infiltration of mononuclear cells; atrophic changes, based on the loss of parietal and chief cells; and hyperplastic changes of foveolar mucus neck cells. In contrast to NTx-TSLPR^{+/+} mice, gastric pH and total gastritis score were significantly elevated in NTx-TSLPR^{-/-} mice (Fig. 3B). These data

suggested that TSLPR deficiency exacerbates inflammation and atrophy of gastric mucosa in AIG.

To further investigate infiltrating cells in the gastric mucosa, these cells were examined by immunohistology. Although AIG in NTx mice is characterized by a marked infiltration of CD4⁺ T cells, the cell numbers of CD4⁺ T cells in the gastric mucosa increased in NTx-TSLPR^{-/-} mice (Fig. 4A, 4B). To confirm this finding, isolated mononuclear cells from the stomach were analyzed by flow cytometry, and CD4⁺ T cell numbers were counted (Fig. 4C, 4D). We found significantly increased numbers of CD4⁺ T cells in the stomachs of NTx-TSLPR^{-/-} mice. Taken together, these data suggested that TSLPR deficiency exacerbates CD4⁺ T cell infiltration in the gastric mucosa in AIG.

TSLPR deficiency induces increased susceptibility to AIG in mice

Finding increased elevated histopathology of AIG, including CD4⁺ T cell infiltration in the gastric mucosa in 12-wk-old NTx-TSLPR^{-/-} mice (Figs. 3, 4), we next examined whether TSLPR deficiency induced earlier onset and/or increased incidence of AIG. The gastric mucosa in mice at the ages of 6, 9, and 12 wk were evaluated histologically. We determined the incidence of AIG as chronic gastric inflammation greater than grade 2, characterized by moderately dense infiltration of mononuclear cells. These infiltrates were further confirmed to be CD4⁺ cells by immunohistological staining using FITC-conjugated anti-CD4. We found that the incidence of AIG in TSLPR^{-/-} NTx mice was significantly higher than that of NTx-TSLPR^{+/+} mice at the age of 6 wk (Fig. 5). These data suggested that TSLPR deficiency induces earlier onset of AIG.

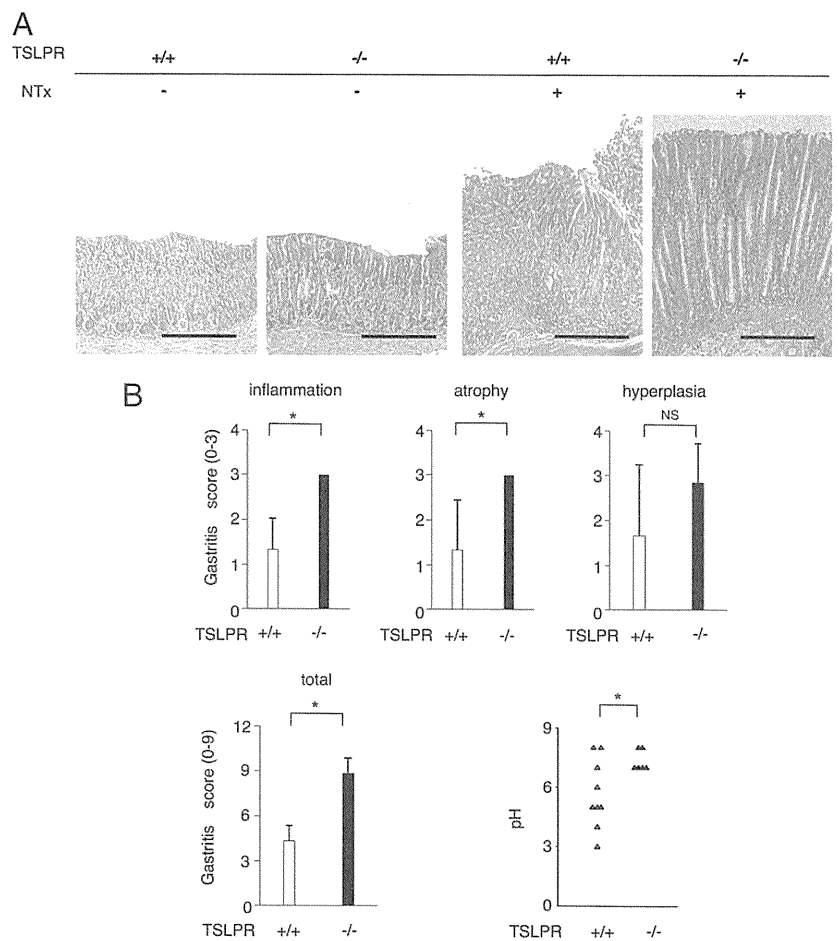


FIGURE 3. TSLPR deficiency exacerbates histopathology in the mouse model of AIG. *A*, Histology of the gastric mucosa in 12-wk-old TSLPR^{+/+} and TSLPR^{-/-} mice, with or without neonatal thymectomy (H&E). Non-NTx TSLPR^{+/+} and TSLPR^{-/-} mice did not exhibit gastritis. Scale bars, 100 μm. *B*, Degree of gastritis in NTx-TSLPR^{+/+} (*n* = 9) and NTx-TSLPR^{-/-} mice (*n* = 7) was determined by a semiquantitative scoring system, as described in *Materials and Methods* (upper panels and lower left panel). Data are presented as mean and SD. Lower left panel, Local pH in the corpus area of the stomach. Each symbol represents an individual mouse. **p* < 0.05, Student *t* test for unpaired data.

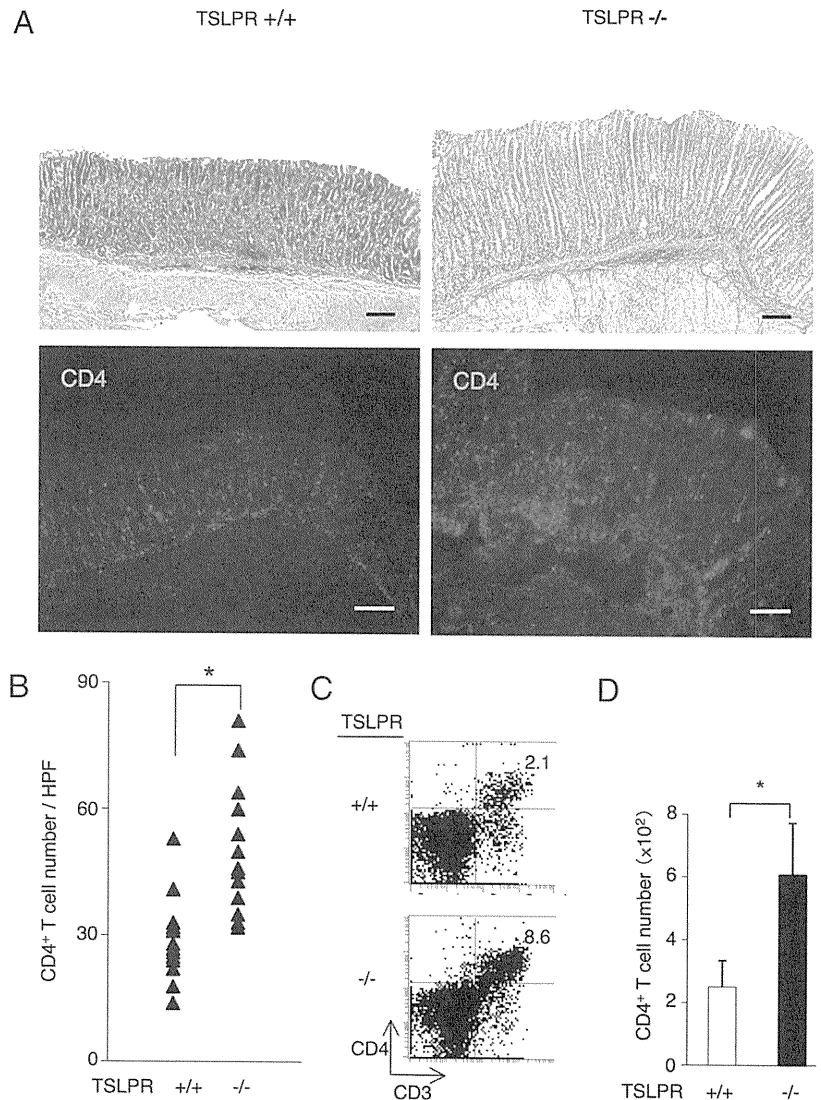


FIGURE 4. TSLPR deficiency exaggerates CD4⁺ T cell infiltration in the gastric mucosa in the mouse model of AIG. *A*, Fluorescence immunohistology of the stomach, using FITC-anti-CD4, in 12 wk-old NTx-TSLPR^{+/+} and NTx-TSLPR^{-/-} mice (*lower panels*). Histologic findings using H&E in the same mice are shown in the *upper panels*. Scale bars, 100 μ m. *B*, Numbers of CD4⁺ T cells in the gastric mucosa. Each symbol represents an individual mouse. **p* < 0.05, Student *t* test for unpaired data. *C* and *D*, Isolated mononuclear cells from the stomach of 12-wk-old NTx-TSLPR^{+/+} and NTx-TSLPR^{-/-} mice were analyzed by flow cytometry. Cells were stained with allophycocyanin-Cy7-conjugated anti-CD4 and PE-conjugated anti-CD3. *C*, Numbers in quadrants indicate percentage of cells in that gate. Data represent one of five separate experiments. *D*, Numbers of CD4⁺ T cells in the gastric mucosa were calculated using the equation (percentage of CD3⁺CD4⁺ cells in viable cells) \times (number of viable cells). Data are the mean and SD of five separate experiments. **p* < 0.05, Student *t* test for paired comparisons.

TSLPR deficiency exhibits exaggerated Th1 responses

Because inflammatory Th1 responses are critical for the development of AIG, and TSLP regulates Th1-type inflammation in

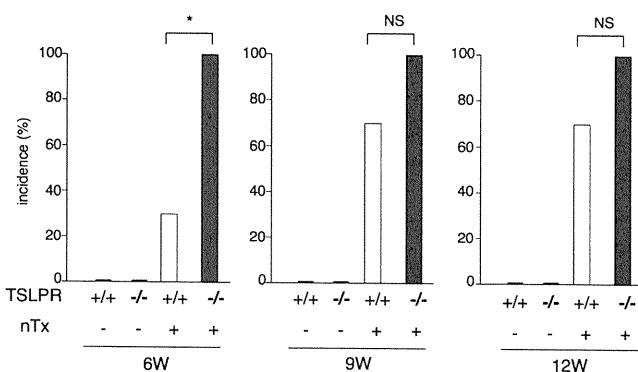
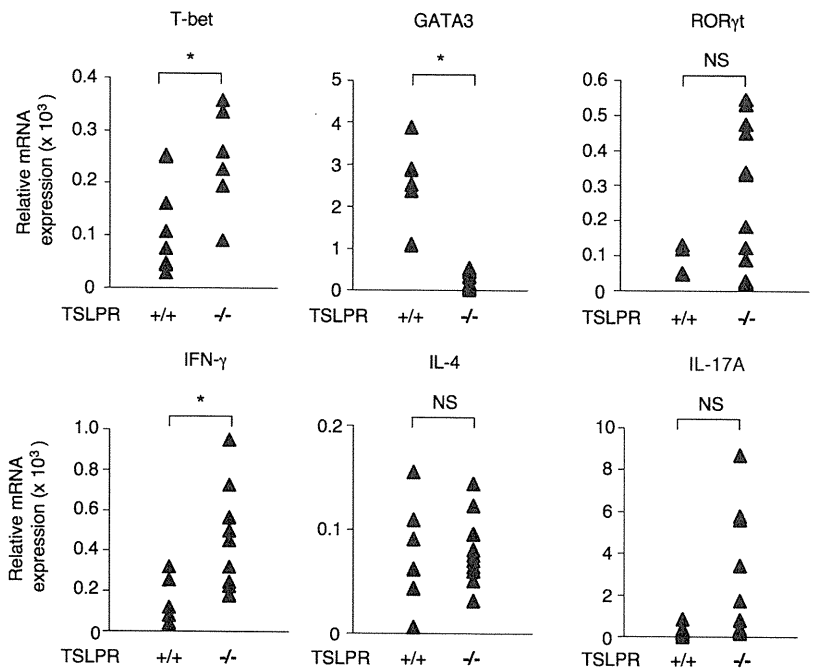


FIGURE 5. TSLPR deficiency induces increased susceptibility to AIG in mice. The gastric mucosa in mice at 6, 9, and 12 wk of age (*n* = 10 in each group) were evaluated histologically. The incidence of AIG was determined by chronic gastric inflammation greater than grade 2, characterized by moderately dense infiltration of mononuclear cells. These infiltrates were confirmed to be CD4⁺ cells by immunohistological staining using FITC-conjugated anti-CD4. **p* < 0.05, Fisher exact test.

mouse models of colitis (11–14, 21), we examined whether TSLPR deficiency enhanced Th1 responses in the inflamed stomach in AIG. We performed real-time quantitative RT-PCR analysis to measure the expression levels of mRNA encoding T cell lineage-specific transcription factors, such as T-bet, GATA3, and ROR γ t, and cytokines, such as IFN- γ , IL-4, and IL-17A. Inflamed gastric tissues of TSLPR^{-/-} NTx mice at 12 wk of age expressed a significantly increased level of mRNA expression of Th1 lineage-specific transcription factor T-bet, together with IFN- γ , in comparison with those in NTx-TSLPR^{+/+} mice (Fig. 6). In contrast, inflamed gastric tissues of NTx-TSLPR^{-/-} mice expressed reduced levels of mRNA expression of Th2 lineage-specific transcription factor GATA3. Levels of mRNA expression of IL-4, as well as Th17-related ROR γ t and IL-17A, were not significantly affected by TSLPR deficiency (Fig. 6). To confirm enhanced expression of IFN- γ by infiltrated CD4⁺ T cells, we performed flow cytometry using intracellular cytokine staining of CD4⁺ T cell infiltrates restimulated with PMA plus ionomycin. We observed significantly increased numbers of CD4⁺ T cells in the stomach of NTx-TSLPR^{-/-} mice (Fig. 4). In addition, we found significantly increased percentages of IFN- γ -expressing cells in gastric CD4⁺ T cells of NTx-TSLPR^{-/-} mice (Fig. 7A). Taken together, these data suggested that TSLPR deficiency enhances Th1 responses in the inflamed gastric tissues in AIG.

FIGURE 6. TSLPR deficiency exhibits exaggerated Th1 responses. Real-time quantitative RT-PCR analysis was used to measure the expression levels of mRNA encoding T cell lineage-specific transcription factors, such as T-bet, GATA3, or ROR γ t, and cytokines, such as IFN- γ , IL-4, and IL-17A, in the stomach of 12-wk-old NTx-TSLPR^{+/+} or NTx-TSLPR^{-/-} mice. Each symbol represents an individual mouse. **p* < 0.05, Student *t* test for unpaired data.



TSLPR deficiency induces enhanced IL-12/23p40 production by DCs in GLN

In mice, TSLP was reported to negatively regulate production of IL-12/23p40 by DCs *in vivo* and *in vitro* and to suppress Th1 responses (21). In AIG-bearing mice, the draining lymph node of the stomach GLN is the induction site for activation of autoreactive CD4⁺ T cells by DCs capturing gastric tissue-specific self-Ag (29). Next, we examined whether TSLP deficiency may enhance DC activation to produce IL-12/23p40 in GLNs. Anti-mouse IL-12/23p40 Abs (clones C15.6 and C17.8) can react with both free and complexed heterodimer p70 forms of the p40 subunit of mouse IL-12, and they have been used to enumerate IL-12-producing cells (21, 30, 31). We isolated cells from the GLN, MLN, and spleen and enumerated IL-12-producing cells using anti-mouse IL-12/23p40 by flow cytometry. In contrast to NTx-TSLPR^{+/+} mice, NTx-TSLPR^{-/-} mice showed significantly increased percentages of IL-12/23p40⁺ cells in CD11c⁺CD11b^{+/−} DCs isolated from the GLN, MLN, and spleen.

In addition, these increased percentages were observed in CD11c⁺CD11b^{+/−} DCs but not in CD11c[−]CD11b⁺ macrophages (Fig. 7*B*). These data suggested that TSLPR deficiency induced enhanced IL-12/23p40 production by DCs in the GLN, the induction sites for activation of autoreactive CD4⁺ T cells in AIG-bearing mice.

Discussion

In the current study, we demonstrated that TSLP was expressed in the inflamed stomach of AIG-bearing mice and that TSLPR deficiency enhanced production of anti-parietal cell Abs. Importantly, NTx-TSLPR^{-/-} mice exhibited early onset of AIG and exaggerated inflammation of gastric mucosa, with enhanced Th1 responses. These data suggested that TSLPR-mediated signaling negatively regulates development of Th1-dependent autoimmunity in the gastric mucosa.

We showed in this study that inflamed gastric tissues in AIG-bearing mice exhibited increased levels of expression of TSLP.

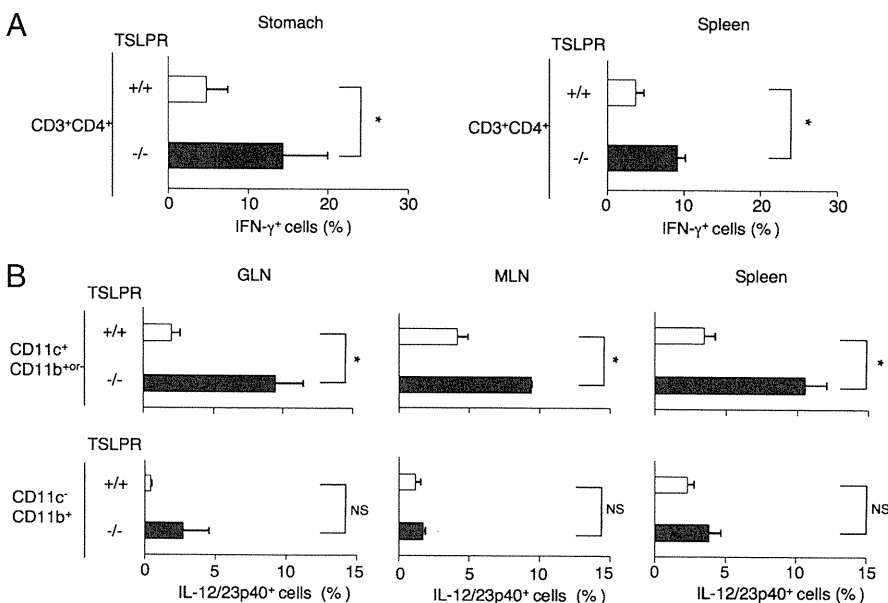


FIGURE 7. TSLPR deficiency enhances production of IFN- γ and IL-12/23p40. *A*, Intracellular cytokine staining of CD3⁺CD4⁺ T cells in the stomach and spleen of 12-wk-old NTx-TSLPR^{+/+} and NTx-TSLPR^{-/-} mice. The cells were isolated from stomachs and spleens and then stimulated with PMA plus ionomycin. Percentages of IFN- γ ⁺ cells in CD3⁺CD4⁺ T cells are shown. *B*, Intracellular cytokine staining of CD11c⁺CD11b^{+/−} cells and CD11[−]CD11b⁺ cells in the GLN, MLN, and spleen of 12-wk-old NTx-TSLPR^{+/+} and NTx-TSLPR^{-/-} mice. Percentages of IL-12/23p40⁺ cells in indicated cells are shown. Data are the mean and SD of five separate experiments. **p* < 0.05, Student *t* test for paired comparisons.

In a previous study in mice, mRNA expression of TSLP could be detected throughout the small and large intestines (20, 21). In patients with eosinophilic esophagitis, mRNA expression of TSLP in the inflamed esophageal tissues was significantly overexpressed (18, 19). TSLP expression was also found in gastric mucosal lesions from patients with *Helicobacter pylori*-induced chronic gastritis and *H. pylori* colonization-induced TSLP production in gastric epithelial cells (27). In the intestine, mRNA expression of TSLP was overexpressed in patients with ulcerative colitis, and proinflammatory cytokines induced overexpression of TSLP in colonic epithelial cells (32, 33). Therefore, TSLP can be expressed in the intestinal tract throughout the esophagus, stomach, and small and large intestines, both in humans and mice, suggesting that TSLP is involved in various immune responses in the gastrointestinal tract.

We also demonstrated in this study that TSLPR deficiency induced early onset of AIG and that the increased susceptibility was associated with exacerbated histopathology accompanied by enhanced Th1 responses. In addition, in AIG-bearing mice, TSLP negatively regulated production of IL-12/23p40 by DCs, as described (21). Therefore, these data suggested that TSLP expression in inflamed mucosa negatively modulates DC activation to promote Th1-type autoimmunity in the stomach. In mice, TSLP is involved in allergic diarrhea and Th2-type intestinal immunity against helminth infection, and it regulates Th1-type inflammation in colitis induced by dextran sodium sulfate (17, 21). In humans, TSLP is a candidate gene critically involved in susceptibility to eosinophilic esophagitis. TSLP overexpression is thought to be involved in *H. pylori*-induced chronic gastritis with formation of B cell follicles (18, 27). In addition, mRNA expression of TSLP was significantly reduced in patients with Crohn's disease, whereas TSLP was overexpressed in patients with ulcerative colitis (32, 33). Taking these results together, although TSLP is deeply involved in the pathophysiology of some allergic or Th2-dependent inflammatory conditions, its primary role in physiological conditions of the gastrointestinal tract may be to maintain intestinal immune homeostasis, including regulation of autoimmunity.

Although inflammatory Th1 responses induced by autoreactive CD4⁺ T cells have the potential to develop AIG in any mice, various regulatory mechanisms in normal mice suppress the development of AIG. NTx-BALB/c mice possess disease-relevant Tregs, but these cannot prevent AIG development (7–9), suggesting that Tregs are critically involved in negatively regulating the development of AIG. In addition, programmed cell death 1 (PD-1) provides negative costimulation to lymphocytes. PD-1-deficient BALB/c mice spontaneously develop AIG (25, 34), suggesting that PD-1-mediated signaling is critical for the negative regulation of AIG development. These regulatory mechanisms are primarily involved in suppressing the development of AIG, whereas although TSLP may be secondarily induced after triggering inflammation of AIG, induced TSLP did not regress the inflammation of AIG. However, it is not clear whether TSLP might have a regulatory function if there were more of it. Further studies are required to ascertain whether TSLP might be used as a therapeutic target for treating patients with AIG.

A previous report showed that adding recombinant TSLP enhanced the proliferation capacity of TCR-stimulated CD4⁺ T cells in vitro and that CD4⁺ T cells from TSLPR-deficient mice expanded less efficiently than did CD4⁺ T cells from wild-type mice in irradiated γ c/Rag2-deficient hosts (35). However, we found that infiltrating mononuclear cells in the gastric mucosa of NTx-TSLPR^{-/-} mice were also mainly CD4⁺ T cells and that TSLPR deficiency increased the cell numbers of CD4⁺ T cells in the

gastric mucosa (Fig. 5). These data suggested that the enhanced proliferative capacity of CD4⁺ T cells in a Th1-dominant autoimmune setting may overcome a deficiency of direct action of TSLP on CD4⁺ T cells.

In adult NTx mice, CD4⁺Foxp3⁺ Tregs were observed in the periphery, as described (7–9). We also found that 12-wk-old NTx-TSLPR^{-/-} mice had a number of CD4⁺Foxp3⁺ Tregs in the periphery, comparable to those found in NTx-TSLPR^{+/+} mice (data not shown). These findings suggested that it is not likely that impaired generation and expansion of induced Tregs enhance CD4⁺ T cell infiltration in the gastric mucosa of NTx-TSLPR^{-/-} mice.

In conclusion, we demonstrated that TSLP was expressed in the inflamed stomach of AIG-bearing mice and that TSLPR deficiency both increased susceptibility to AIG and exacerbated its severity. These data suggested that TSLPR-mediated signaling negatively regulates organ-specific Th1-dependent autoimmunity in the gastric mucosa.

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Disclosures

The authors have no financial conflicts of interest.

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