が目安とされていたが，最近では，RA疑いの時点でDMARDsを開始する傾向がある．欧米のリウマチ医のグループは初期RAを強く疑う所見として，3カ所以上の腫脹，MCP/MTP関節炎，30分以上の朝のこわばりを挙げている[7]．滑膜炎がMCP, MTP関節のようにRAに特徴的な部位にある場合，MRI所見（増殖性滑膜炎，骨髄浮腫[8]），RFや抗CCP抗体などを参考にDMARD治療を開始して良いと考える．

2. 抗リウマチ薬の選び方

1）Strong DMARDs

本邦で使用可能な抗リウマチ薬は表1のごとく12種類ある．FelsonはDMARDsの有効性を検討した臨床試験の成績をmeta-analysisした結果，MTX，SASP，D-ペニシラミン，注射金剤が経口金剤に比べて有意に優れていたことから[9]治療効果の高い薬剤をstrong DMARDsと位置付けた．これに対して経口金剤や抗マラリア薬をmild DMARDsとした．ACR改善率や本邦臨床試験で中等度改善以上が50%以上を示し，無作為化試験で骨破壊進行抑制効果が確認されている薬剤をstrong DMARDsとすれば，これらがRA治療の中心となる（表1）．

2）DMARDs選択の際のポイント

DMARDsの選択にあたってはRA疾患活動性，骨破壊の有無，罹病期間を考慮する．薬剤としては効果の発現の速さ，有効率，骨破壊進行抑制効果が考慮される．関節破壊の予後不良因子として，高い疾患活動性（DAS＞5.1），大関節障害，リウマトイド因子陽性，既骨破壊，HLA-DR4（shared epitope）陽性が報告されている[10]．実際の診療では，HLA-DR4の検査など遺伝学的検査は簡単にできないので，RA活動性，骨破壊の有無が重要な点であり，加えて罹病期間，年齢や臓器合併症を考慮する．活動性評価にはDAS（disease activity score）が使用しやすい．フランスリウマチ学会より示された極初期RAに対する第1DMARDの選択基準では，DASにより疾患活動性を3段階に分け，さらに骨破壊やRFの有無でDMARDsの決定を行う[11]．活動性が高い症例，すでに骨破壊がある症例では骨破壊の進行が速いので，有効率や骨破壊進行抑制効果が優れていて，効果の発現が速い

表1 本邦における抗リウマチ薬の分類と有効性

分類	薬剤（商品名）	有効率	効果発現	骨破壊進行抑制効果	免疫抑制作用
strong DMARDs	金チオリンゴ酸ナトリウム（シオゾール）	約40%以上の有効率	遅	◎	(−)
	ブシラミン（リマチル）	≧中等度善：40%＊ ACR20:48%	中	○	(−)
	D-ペニシラミン（メタルカプターゼ）	≧中等度善：65%（300～600 mg/日）	中	○	(−)
	メトトレキサート（リウマトレックス）	≧中等度善：60%（6 mg） 64%（9 mg）	速	◎	◎
	サラゾスルファピリジン（アザルフィジンEN）	≧中等度善：58%＊	速～中	◎	(−)
	レフルノミド（アラバ）	ACR 20：52.6%	速	◎	◎
	タクロリムス（プログラフ）	ACR20:49%, ACR50:27.5%	速	No data	◎
	イグラチモド：T-514（未発売）（ケアラム，コルベット）	ACR 20：53.4～62.5%	中	○	(−)
mild DMARDs	オーラノフィン（リドーラ）	≧中等度善：40%＊	遅	No data	(−)
	アクタリット（オークル，モーバー）	≧中等度善：37%＊	遅	No data	(−)
	ロベンザリット（カルフェニール）	≧中等度善：33%＊	遅	No data	(−)
	ミゾリビン（ブレディニン）	全般的改善度≧改善：24%	遅	No data	○

MTX, LEFあるいは併用療法を選ぶ．本邦で可能なDMARDs併用療法としては，MTXとブシラミンや注射金剤との併用が単剤より優れている事が証明されている．また，罹病期間が長い症例では1年未満の症例に比べてDMARD有効率が低いので，MTXなど有効性が高い薬剤を選択する．筆者はDMARD選択の一応の目安として図1のように考えている．

3. 抗リウマチ薬によるRAの治療目標

DMARDの治療効果は短期的に判定しなければならないので，症状・徴候の改善度を中心としたACR改善率（20%, 50%, 70%, 90%）あるいはDASが簡便で使いやすい．長期的には骨破壊進行の有無を関節単純X線撮影で評価する．DASやACR改善率とX線写真上の関節破壊の進行抑制効果には相関がある．筆者の検討では，DAS＜2.6の寛解例，腫脹関節が消失した例では，1年後に骨破壊の進行はほとんどみられない．また，DAS＜3.2の低活動性やACR70%以上の著明改善例では骨破壊進行例は少ないので，治療目標の目安になる[12]．若年の早期RA症例では寛解を目標に治療する．しかし，高齢者や内臓合併症のある症例では，強力な治療は重篤な副作用をまねく危険があるのでACR50%改善やDAS中等度改善を目安にする．

4. 副作用への対応

DMARDsの副作用は多様で，時に重篤になる．特に高齢者や腎機能低下例では細心の注意が必要である．以下に副作用対策のポイントを挙げる．①頻度が多い副作用と重篤な副作用を患者に説明する．②過敏反応（hypersensitivity）と毒性（toxicity）によって起きる副作用では発現時期が違う事を理解する．前者はMTX肺臓炎やSASPの皮疹，血球減少のように投与開始後6カ月以内に起きやすい．後者は用量依存性があり発現時期が一定していない．③危険因子のある例や禁忌症例に投与しない．④MTX，LEF，タクロリ

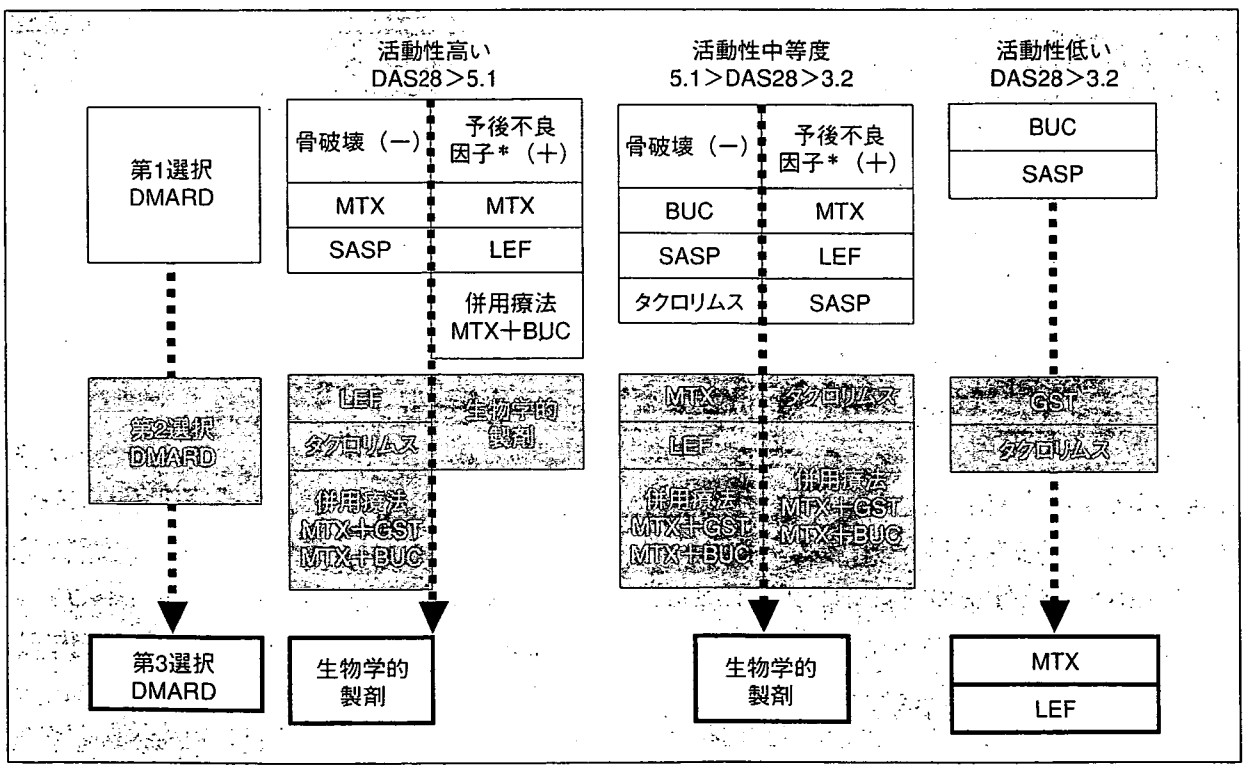

図1 抗リウマチ薬選択の目安

*：骨破壊＋，RF陽性，罹病期間＞5年．

表2 主な抗リウマチ薬の主な副作用

薬剤と剤型	禁忌・慎重投与	副作用		
		頻度が多い	重篤	特殊
メトトレキサート メソトレキセート®錠 (2.5 mg) RA 未承認 リウマトレックス®カプセル (2 mg) *	(禁) 肝疾患 HB, HCV 陽性例 腎不全 (慎) 間質性肺病変, アルコール常飲者	肝機能異常 口内炎 皮疹 胃腸障害	間質性肺炎 骨髄障害 肝線維化 日和見感染	リンパ増殖疾患 催奇形性 結節症 血管炎
レフルノミド アラバ®錠 (100, 20, 10 mg)	(禁) 間質性肺病変 (慎) 高齢者, 低蛋白血症, HB,HCV 陽性例, 耐糖能異常	下痢, 脱毛 皮疹	間質性肺炎 骨髄障害, 日和見感染 Stevens-Johnson 症候群	高血圧
サラゾスルファピリジン アザルフィジン EN®錠 (250, 500 mg)	(禁) サルファ剤アレルギー (慎) 多剤薬剤アレルギー	皮疹 肝障害 消化器症状	骨髄障害 Stevens-Johnson 症候群	薬剤性過敏症候群: DIHS =伝染性単核球症様症状
ブシラミン リマチル®錠 (50, 100 mg)	(禁) 腎障害	皮疹, 味覚障害 口内炎, 蛋白尿	ネフローゼ 間質性肺炎	爪の変形, 黄色爪 自己免疫疾患誘発
D-ペニシラミン メタルカプターゼ®カプセル (100 mg)	(禁) 腎障害	皮疹, 味覚障害 口内炎 蛋白尿	ネフローゼ 骨髄障害	自己免疫疾患誘発 (筋炎, 筋無力症 ANCA 血管炎)
注射金剤 シオゾール®(10 mg, 25 mg)	(慎) 多剤薬剤アレルギー, 腎障害, 間質性肺病変	皮疹	蛋白尿 間質性肺炎	
オーラノフィン リドーラ®錠 (3 mg)		下痢, 軟便, 腹痛		
アクタリット オークル®錠 (100 mg) モーバー®錠 (100 mg)		皮疹, めまい	骨髄障害 腎障害	
ロベンザリット カルフェニール® (40, 80 mg)	(禁) 腎障害	血清クレアチニン上昇	腎不全	
ミゾリビン ブレディニン®錠 (25, 50 mg)		胃腸障害 高尿酸血症	骨髄障害	催奇形性
タクロリムス (FK-506) プログラフ®カプセル (0.5, 1, 5 mg)	(禁) 腎不全 HB, HCV 陽性例 (慎) 腎機能低下, 高血圧, 耐糖能異常 心疾患	BUN, Cr, カリウム 尿酸上昇 高血糖 振戦, 頭痛	腎障害, 腎不全 中枢神経障害 日和見感染 不整脈, 心不全	相互作用多し

ムスのような免疫抑制作用のある薬剤（表1）では日和見感染が起こりうることを念頭に置く．⑤直ちに中止して治療，救命法が必要な副作用を理解する．一般に過敏症による副作用は直ちに中止し，MTX や LEF の重篤な副作用のように救命療法（ロイコボリン®やクエストラン®）が必要な薬剤は速やかに行う．各 DMARDs の副作用の対策を表2にまとめる．

免疫抑制薬

本邦で使用可能な免疫抑制薬は表3に示す7剤であるがミコフェノール酸モフェチルは保険適用上使用しにくい．ここでは，RA 以外の疾患・病態別の適応，注意すべき副作用について解説する．

1. 作用機序

免疫抑制薬は作用機序によりアルキル化薬，核酸（プリン/ピリミジン）代謝拮抗薬，カルシニューリン阻害薬の3種類に大別される．詳細は表3に解説する[13]．

2. 各疾患における免疫抑制薬の適応（表4）

（1）全身性エリテマトーデス（SLE）：SLE 患者では約30%の症例に免疫抑制薬が使用されているとの報告がある[14]．目的は難治性病態の治療とステロイド減量目的である．

表3 免疫抑制薬の作用機序

薬剤	分類	作用機序	血中半減期
シクロホスファミド	アルキル化薬	活性体 phosphoramide mustard が DNA とクロスリンクし，DNA の複製を阻害 T 細胞，B 細胞ともに抑制．抗炎症作用あり	2～8 時間 8.7 時間 (mustard)
メトトレキサート	プリン/ピリミジン代謝拮抗薬	化学構造は葉酸誘導体 葉酸代謝の key enzyme ジヒドロ葉酸（DHF）還元酵素（DHFR）と結合・抑制しチミジン合成を阻害しプリン，ピリミジン合成を抑制の結果，DNA，RNA 合成を抑える ホモシステイン→メチオニンの変換阻害による蛋白合成低下 アデノシンの放出促進による抗炎症作用	1.5～4 時間 (分布相) 8～15 時間 (消失相)
アザチオプリン	プリン代謝拮抗薬	6-mercaptopurine（6MP）のイミダゾール誘導体 体内で 6MP に変換，細胞内で inosine monophosphate の類似体，6MP riibonucleotide を形成しプリン合成を阻害．DNA，RNA 合成に関わる他の酵素もフィードバック機構で抑制．T 細胞，B 細胞増殖を抑制	1.7 時間 1.2～1.5 時間 (6MP)
ミゾリビン		イミダゾール系プリン誘導体 細胞内でリン酸化され，高分子核酸中には取り込まれないで，プリン合成系の IMPDH（inosine monophosphate dehydrogenase），GMPS（guanosine monophosphate synthetase）を拮抗阻害し，DNA 合成を抑制．salvage 合成経路には影響しない T，B 細胞ともに抑制	2.2 時間
ミコフェノール酸モフェチル		体内で速やかに加水分解し活性体のミコフェノール酸になる DNA の構成成分であるプリンの *de novo* 合成経路の key enzyme, IMPDH の強力な可逆的，非競合的阻害薬 T 細胞増殖抑制，B 細胞抗体産生抑制．プリン合成に，より salvage 合成経路を使用する骨髄細胞の抑制は少ない	16 時間
シクロスポリン	カルシニューリン阻害薬 (calcineurin inhibitors)	特異的結合蛋白（イムノフィリン）であるシクロフィリン（cyclophilin）や FK 結合蛋白（FKBP）に結合し，リン酸化酵素であるカルシニューリンを阻害．その結果，*IL-2* 遺伝子の転写を抑制し特異的に T 細胞を抑制	10～30 時間
タクロリムス(FK-506)			7～20 時間

適応となる難治性病態として重症ループス腎炎，中枢神経症状，血管炎合併が挙げられる．難治性ループス腎炎（WHO Ⅲ，Ⅳ，Ⅴ＋Ⅳ型）はステロイド単独治療では腎不全への進行する症例が多く，CY 間欠的点滴静注の導入療法としての有効性が NIH の報告や Euro-Lupus Nephritis trial で示されている[15～17]．また，最近，ミコフェノール酸モフェチル（MMF）の有効性が導入・維持療法ともに示されている[18, 19]．MMF は本邦では使用しづらいが，MMF と同じ作用機序を持つミゾリビン（MZB）も高用量や 1 回投与法により血中濃度を上げれば有効である可能性がある．また，CyA や維持療法における AZ の有効性も報告されている．膜性腎炎に対しても免疫抑制薬併用の有用性が示されているが今後，RCT での検討が必要である．他の臓器障害では難治性中枢神経症状が良い適応である．ステロイドが無効の中枢神経ループス（器質脳症候群，痙攣，脳血管障害，精神症状）に intravenous pulse cyclophosphamid（IV-CY）療法が有効であったとの報告がある[20, 21]．MTX は非腎症 SLE に対する RCT で，関節・皮膚症状に対する有用性が示されている[22]．

(2) 多発性筋炎/皮膚筋炎（PM/DM）：免疫抑制薬の適応はステロイド不応性の難治性筋炎と間質性肺病変，DM の皮膚潰瘍や血管炎である．難治性筋炎に対しては，古くからアザチオプリン（AZ），シクロホスファミド（CY），MTX が使用されている．筋症状や検査所見の改善率や改善までの期間が免疫抑制薬併用の方が優れているという成績はないが，ステロイドの減量効果は示されている．近年，CyA も MTX と同等の効果があるとい

表4 疾患/病態別にみた膠原病における免疫抑制薬の適応

疾患	病態	免疫抑制薬
関節リウマチ	血管炎	末梢型：AZ　全身型:CY, IV-CY
	間質性肺病変	AZ, CY, CyA, FK
成人 Still 病	慢性関節炎，ステロイドの減量目的	MTX
全身性エリテマトーデス (SLE)	慢性持続性関節炎	MTX
	増殖性腎炎（WHO Ⅲ，Ⅳ，Ⅳ＋Ⅴ）導入療法 維持療法	IV-CY, MMF, CyA MMF, AZ
	膜性腎炎（WHO Ⅴ）	AZ
	CNS	IV-CY
	血管炎	AZ, CY (IV-CY)
	脂肪織炎,血球貪食症候群	CyA
	非腎症 SLE のステロイド減量目的	AZ, MZ
全身性強皮症	間質性肺病変 非高血圧性腎クリーゼ（MPO-ANCA 陽性例）	CY, IV-CY, CyA IV-CY
多発性筋炎/皮膚筋炎	ステロイド反応不良，不応 間質性肺病変 皮膚潰瘍，血管炎 皮膚症状	MTX, AZ, CY, CyA CyA, CY CY MMF
血管炎	古典的結節性多発動脈炎	IV-CY
	顕微鏡的多発血管炎：全身性血管炎型，肺腎型，RPGN 型 腎限局型，肺線維症型，その他軽症型 Wegener 肉芽腫症 Churg-Strauss 症候群（多臓器障害，運動神経障害）	CY, IV-CY AZ（IV-CY） CY, IV-CY CY, IV-CY
	大動脈炎症候群（ステロイド減量目的）	MTX
Behçet 病	難治性反復性ブドウ膜炎 慢性進行性の中枢神経症状 難治性腸管 Behçet 病	CyA MTX MTX, AZ

MTX: methotrexate, AZ: azathioprine, MZ: mizoribin, CyA: cyclosporine, FK: tacrolimus, CY: cyclophosphamide
IV-CY: intravenous pulse cyclophosphamide, MMF: mycophenolate mofetil

われる[23]．PM/DM，特に amyopathic DM に合併する急速進行性間質性肺病変に対しては，現在までのところ有効性が確立した免疫抑制薬はないが IV-CY やシクロスポリン (CyA)，タクロリムス (FK) の有効性を示唆する報告もある[24]．皮膚潰瘍や皮膚血管炎を合併する DM には CY が有効である．

(3) 全身性強皮症 (SSc)：急性間質性肺炎（活動性肺胞炎）に対して，IV-CY が有効であったとの報告がみられる[25]．CyA が単剤あるいはプロスタグランジン製剤併用で皮膚硬化や末梢循環障害，食道病変に対して有効性を示す成績が報告されている[26]．最近，強皮症を伴う早期の肺胞炎に対して，MMF と少量ステロイド併用が肺機能と画像所見を改善させたとの報告がある[27]．MPO-ANCA 陽性の非高血圧性腎クリーゼでは CY の有効性が期待できる．

(4) 血管炎症候群：古典的結節性多発動脈炎や ANCA 関連血管炎に対しては，CY の有効性が知られている．経口連日投与と IV-CY 療法の両者を比較した成績もあるが，効果は同程度で，副作用や死亡率については IV-CY 療法が優れているとの報告が多い[28,29]．しかし，Wegener 肉芽腫症に対して経口投与と IV-CY 療法の効果を比較した成績では，寛解導入率に差はなかったが，経口法の方が再燃率は低かった[30]．

表5　主な免疫抑制薬の用法と副作用

薬剤と剤型	一般的投与量	保険適用	慎重投与	副作用			
				頻度が多い	重篤	特殊	モニタリング
メトトレキサート メソトレキセート®錠 (2.5 mg) リウマトレックス®カプセル (2 mg)	5～15 mg, 週1～2日 4～8 mg, 週1～2日(保険上)	抗癌薬 RA	肝疾患 (HB, HCV＋例) 間質性肺病変 アルコール 常飲者	肝機能異常 口内炎 皮疹 胃腸障害	間質性肺炎 骨髄障害 肝線維化	リンパ増殖疾患 催奇形性 結節症 血管炎	胸部X線 CBC 肝機能 (AST, ALT, Alb)
アザチオプリン イムラン®錠(50 mg) アザニン®錠(50 mg)	1.5～2 mg/k体重 (75～100 mg/日) 分1～2	腎移植	肝疾患 (HB, HCV＋例) アロプリノール使用例	肝障害 胃腸障害	骨髄障害 発癌性 易感染性	催奇形性	CBC 肝機能
シクロホスファミド エンドキサン®末 エンドキサンP®(50 mg) 注射用 (100, 500 mg)	1.5～2 mg/kg体重 (75～100 mg/日), 分1～2 点滴静注 (500～750 mg/回, 月1回)	抗癌薬	妊娠希望の若年女性 排尿障害(前立腺疾患など残尿あり)	胃腸障害 白血球数減少 脱毛	骨髄障害 発癌性 出血性膀胱炎 易感染性	生殖機能障害 催奇形性	検尿(沈渣) CBC 肝機能
ミゾリビン ブレディニン®錠 (25, 50 mg)	150 mg, 分3	腎移植 RA ループス腎炎 ネフローゼ		胃腸障害		催奇形性	CBC 肝機能 腎機能, 尿酸
シクロスポリン サンディミュン®カプセル ネオーラル®カプセル (25, 50 mg) 内用液10% 注射液0.25 g	1～4 mg/kg体重 分1～2	臓器移植 ネフローゼ, 乾癬 Behçet病 赤芽球癆 再生不良性貧血	腎機能低下 高血圧 耐糖能異常	多毛 歯肉肥厚 しびれ BUN, Cr上昇 肝機能異常	腎障害, 腎不全 急性膵炎 易感染性	筋症, 末梢神経障害 神経Behçet誘発 相互作用多し (マクロライド, アゾール系抗生剤, Ca拮抗薬, 抗痙攣薬)	CBC 腎機能 (BMG, カリウム) 肝機能, 血糖 血圧
タクロリムス(FK-506) プログラフ®カプセル (0.5, 1, 5 mg)	1.5～5 mg/日 分1	臓器移植 重症筋無力症 RA	腎機能低下 高血圧 耐糖能異常 心疾患	BUN, Cr, カリウム 尿酸上昇 高血糖 振戦, 頭痛	腎障害, 腎不全 中枢神経障害 易感染性 不整脈, 心不全	相互作用多し (CyAに同じ)	CBC 腎機能 肝機能, 血糖 血圧, ECG
ミコフェノール酸モフェチル セルセプト®カプセル (250 mg)	1回1 g, 1日2回 経過で半量	臓器移植		消化器症状 (下痢, 嘔気) 肝機能異常	血球減少症 易感染性	低リン酸血症 催奇形性	CBC 肝機能, 尿酸, 脂質

大動脈炎症候群では，ステロイド抵抗性あるいは再燃を繰り返す症例でMTXのステロイドの減量効果が示されている[31]．

(5) Behçet病：難治性再発性のブドウ膜炎や後部ブドウ膜炎に対してCyAの有効性が確立されているが，神経Behçetの誘発に注意が必要である．また，神経Behçetのうち慢性進行性の痴呆様精神症状に対してMTXの有効性が報告されている[32]．

3. 副作用への対応

免疫抑制薬の共通の副作用は易感染性である．特に，高齢者や肺合併症のある症例では注意が必要である．細胞毒性のある薬剤は骨髄障害，脱毛，長期使用による発がん性が問題となる．カルシニューリン阻害薬は副作用や薬剤相互作用に特徴があり，またTDMも重要である．詳細は表5を参照されたい．

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著者連絡先

(〒259-1193)
神奈川県伊勢原市下糟屋 143
東海大学医学部内科学系リウマチ内科学
鈴木康夫
[E-mail: y3suzuki@is.icc.u-tokai.ac.jp]

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Clinical and Immunogenetic Features of Patients With Autoantibodies to Asparaginyl–Transfer RNA Synthetase

Michito Hirakata,[1] Akira Suwa,[2] Tetsuya Takada,[1] Shinji Sato,[1] Sonoko Nagai,[3] Ekkehard Genth,[4] Yeong W. Song,[5] Tsuneyo Mimori,[3] and Ira N. Targoff[6]

Objective. **We have previously described anti-KS autoantibodies and provided evidence that they are directed against asparaginyl–transfer RNA (tRNA) synthetase (AsnRS). The aim of the present study was to identify patients with anti-AsnRS autoantibodies and elucidate the clinical significance of this sixth antisynthetase antibody. In particular, we studied whether it was associated with the syndrome of myositis (polymyositis or dermatomyositis [DM]), interstitial lung disease (ILD), arthritis, and other features that had been previously associated with the 5 other anti–aminoacyl–tRNA synthetase autoantibodies.**

Methods. **More than 2,500 sera from patients with connective tissue disease (including myositis and ILD) and controls were examined for anti-AsnRS autoantibodies by immunoprecipitation (IP). Positive and control sera were tested for the ability to inhibit AsnRS by preincubation of the enzyme source with the serum. The HLA class II (DRB1, DQA1, DQB1, DPB1) alleles were identified from restriction fragment length polymorphism of polymerase chain reaction–amplified genomic DNA.**

Results. **Anti-AsnRS antibodies were identified in the sera of 8 patients (5 Japanese, 1 American, 1 German, and 1 Korean) by IP of the same distinctive set of tRNA and protein that differed from those precipitated by the other 5 antisynthetases, and these antibodies showed specific inhibition of AsnRS activity. Two of these patients had DM, but 7 of 8 (88%) had ILD. Four patients (50%) had arthritis, and 1 had Raynaud's phenomenon. This antisynthetase was very rare among myositis patients (present in 0% of Japanese myositis patients), but it was found in 3% of Japanese ILD patients. Thus, most patients with anti-AsnRS had chronic ILD with or without features of connective tissue disease. Interestingly, all 4 Japanese patients tested had DR2 (DRB1*1501/1502), compared with 33% of healthy controls.**

Conclusion. **These results indicate that anti-AsnRS autoantibodies, like anti–alanyl–tRNA synthetase autoantibodies, have a stronger association with ILD than with myositis and may be associated with the DR2 phenotype.**

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[1]Michito Hirakata, MD, Tetsuya Takada, MD, Shinji Sato, MD: Keio University School of Medicine, Tokyo, Japan; [2]Akira Suwa, MD: Tokai University School of Medicine, Isehara, Japan; [3]Sonoko Nagai, MD, Tsuneyo Mimori, MD: Kyoto University Graduate School of Medicine, Kyoto, Japan; [4]Ekkehard Genth, MD: Clinic and Research Institute for Rheumatic Diseases Aachen, Aachen, Germany; [5]Yeong W. Song, MD: Seoul National University Hospital, Seoul, Korea; [6]Ira N. Targoff, MD: Veterans Affairs Medical Center, University of Oklahoma Health Sciences Center, and Oklahoma Medical Research Foundation, Oklahoma City.

Dr. Targoff serves as a technical consultant to the Oklahoma Medical Research Foundation Clinical Immunology Laboratory.

Address correspondence and reprint requests to Michito Hirakata, MD, Section of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: mhirakat@sc.itc.keio.ac.jp.

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The aminoacyl–transfer RNA (aminoacyl-tRNA) synthetases are a family of cytoplasmic enzymes that catalyze the formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA and play a crucial role in protein synthesis. Autoantibodies to certain of these synthetases (histidyl–, threonyl–, alanyl–, isoleucyl–, and glycyl–tRNA synthetases) have been identified in patients with inflammatory myopathies (1–6). Among these "antisynthetase autoantibodies," the most common is anti–Jo-1 (anti–histidyl–tRNA synthetase [anti-HisRS]), found in 20% of patients with polymyositis/dermatomyositis (PM/DM) (7–11). Anti–PL-7 (anti–threonyl–tRNA synthetase [anti-ThrRS])

and anti–PL-12 (anti–alanyl–tRNA synthetase [anti-AlaRS]) autoantibodies are less common, found in 3–4% of all patients with PM/DM (4,5,11–13), while anti-OJ (anti–isoleucyl–tRNA synthetase [anti-IleRS]) and anti-EJ (anti–glycyl–tRNA synthetase [anti-GlyRS]) autoantibodies are the least common, occurring in <2% (6,14,15), although the frequency may vary in different populations (16).

Characteristic clinical features have been found in patients with anti-HisRS and other antisynthetase autoantibodies (1,9,10). These features include myositis, interstitial lung disease (ILD), arthritis, Raynaud's phenomenon, fever with exacerbations, and the skin lesion of the fingers referred to as mechanic's hands, and they appear to form a distinct syndrome referred to as the "antisynthetase syndrome" (8–11). Although the similarity of the clinical features associated with different antisynthetases is impressive (17,18), certain differences have been noted, which must be considered preliminary due to the small reported number of patients with non-HisRS antisynthetases (1,9,19). Patients with anti-AlaRS appear to be more likely than those with anti-HisRS to have ILD and/or arthritis either without myositis or with little evidence of muscle disease. Absence of significant myositis over the full disease course in patients with anti-HisRS is rare (<5%), although it may occur. Clinically significant myositis was seen in 60% of US patients with anti-AlaRS (13), whereas none of 6 Japanese patients with anti-AlaRS autoantibodies fulfilled criteria for myositis (20). Among patients with anti-IleRS, 2 of 10 had ILD without evidence of myositis, and 1 had ILD with subclinical myositis (14). In addition, antisynthetases may occur in either PM or DM, but PM is usually more common with anti-HisRS (10,16,21), and DM is usually more common with other antisynthetases, especially anti-GlyRS (15,22).

We recently described anti-KS autoantibodies and provided evidence that the KS antigen is asparaginyl–tRNA synthetase (AsnRS) (23). This sixth antisynthetase was found in sera from 3 patients with ILD and/or inflammatory arthritis without evidence of myositis. It immunoprecipitated a 65-kd protein and a unique tRNA that was distinct from that precipitated by any previously described antisynthetase or other reported tRNA-related antibody. Each of the 3 sera and their IgG fractions showed significant inhibition of AsnRS activity, but did not inhibit any of the other 19 aminoacyl–tRNA synthetase activities.

In this report, we describe the clinical and immunogenetic features of 5 additional patients with anti-AsnRS autoantibodies, most of whom had the syndrome of ILD with arthritis and/or myositis. Immunoprecipitation (IP) and aminoacylation inhibition studies with sera from these patients provide additional evidence that anti-KS (anti-AsnRS) reacts with asparaginyl–tRNA synthetase.

PATIENTS AND METHODS

Sera. Serum samples from a collection of sera from ~800 patients seen at the current or previous collaborating centers of the authors (Keio University, Tokyo, Japan; Kyoto University, Kyoto, Japan; Seoul National University, Seoul, Korea; Clinic and Research Institute for Rheumatic Diseases Aachen, Aachen, Germany; University of Oklahoma Health Sciences Center, Oklahoma City; National Institutes of Health, Bethesda, MD) or sera referred there for testing were stored at −20°C and were tested for the presence of anti-AsnRS autoantibodies. Sera from the following patients were included: 1) patients with PM or DM according to the criteria described by Bohan and Peter (24,25); 2) patients with a condition suggesting the clinical diagnosis of myositis; 3) patients with ILD who had no evidence of myositis and did not meet criteria for other connective tissue diseases; and 4) patients with serum anticytoplasmic antibodies, regardless of diagnosis. Approximately 1,700 other sera have also been tested, including sera from patients with other conditions including systemic lupus erythematosus, systemic sclerosis, and rheumatoid arthritis, as well as sera from normal subjects. Many of the sera were tested in studies of other autoantibodies. All samples were obtained after the patients gave their informed consent, as approved by the corresponding institutional review boards. Stored sera known to contain autoantibodies against synthetases for histidine, threonine, alanine, glycine, and isoleucine were used as controls.

ILD was considered to be present if an interstitial infiltrate was observed on chest radiography. DM was considered to be present if a heliotrope rash and/or Gottron's papules were observed.

IP. IP from HeLa cell extracts was performed as previously described (6,10). Ten microliters of patient sera was mixed with 2 mg of protein A–Sepharose CL-4B (Pharmacia Biotech, Uppsala, Sweden) in 500 μl of IP buffer (10 m*M* Tris HCl at pH 7.5, 500 m*M* NaCl, 0.1% Nonidet P40 [NP40]) and incubated with end-over-end rotation (Labquake shaker; Lab Industries, Berkeley, CA) for 2 hours at 4°C. The IgG-coated Sepharose was washed 4 times in 500 μl of IP buffer using 10-second spins in a microfuge tube, and resuspended in 400 μl of NET-2 buffer (50 m*M* Tris HCl at pH 7.5, 150 m*M* NaCl, 0.05% NP40).

For analysis of RNAs, this suspension was incubated with 100 μl of extracts, derived from 6×10^6 cells, on the rotator for 2 hours at 4°C. The antigen-bound Sepharose was then collected with a 10-second centrifugation in the microfuge, washed 4 times with NET-2 buffer, and resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 3.0*M* sodium acetate, 30 μl of 10% sodium dodecyl sulfate (SDS), and 300 μl of phenol/chloroform/isoamyl alcohol (50:50:1; containing 0.1% 8-hydroxyquinoline) were added to the Sepharose beads. After agitation in a Vortex mixer and

spinning for 1 minute, RNAs were recovered in the aqueous phase after ethanol precipitation and dissolved in 20 μl of electrophoresis sample buffer, composed of 10*M* urea, 0.025% bromphenol blue, and 0.025% xylene cyanol FF (Bio-Rad, Hercules, CA) in Tris–borate–EDTA buffer (90 m*M* Tris HCl at pH 8.6, 90 m*M* boric acid, and 1 m*M* EDTA). The RNA samples were denatured at 65°C for 5 minutes and then resolved by 7*M* urea–10% polyacrylamide gel electrophoresis (PAGE), with silver staining (Bio-Rad).

For protein studies, antibody-coated Sepharose was mixed with 400 μl of ^{35}S-methionine–labeled HeLa extract derived from 2×10^5 cells and rotated at 4°C for 2 hours. After 4 washes with IP buffer, the Sepharose was resuspended in SDS sample buffer (2% SDS, 10% glycerol, 62.5 m*M* Tris HCl at pH 6.8, 0.005% bromphenol blue). After heating at 90°C for 5 minutes, the proteins were fractionated by 10% SDS-PAGE, enhanced with 0.5*M* sodium salicylate, and dried. Labeled proteins were analyzed by autoradiography.

Aminoacylation. Aminoacylation inhibition reactions were performed as described previously, with minor modification (6,26). Six microliters of HeLa cell extract diluted 1:10 in Tris buffered saline was incubated with 3 μl of a 1:10 dilution of serum for 2 hours at 4°C. This was combined with 17 μl of reaction solution (50 m*M* Tris HCl at pH 7.5, 0.02*M* NaCl, 0.01*M* $MgSO_4$, 1 m*M* dithiothreitol) containing 8 units of yeast tRNA, 3 μl of ^{14}C-asparagine or other ^{3}H-labeled amino acid, and 1 μl of 200 m*M* cold amino acid. Ten-microliter aliquots were tested at 10 minutes and 20 minutes, spotted onto filter paper treated with 5% trichloroacetic acid (TCA), washed 5 times with 5% TCA, then with ethanol, then dried for counting. Results of inhibition testing were expressed as the percent inhibition of the average activity seen with the normal serum included in that experiment, as follows: % inhibition = [(average counts per minute with normal serum) − (cpm with test serum)] × 100/(average cpm with normal serum). Inhibition of >50% compared with the activity with normal serum was considered significant. In previous studies, although nonspecific effects on aminoacylation reactions by serum were common, nonspecific inhibition was usually <25%, and inhibition >50% reliably reflected specific antibody effects (6,7,12,13,26).

DNA typing of the HLA class II (DRB1, DQA1, DQB1, DPB1) alleles by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP). Genomic DNA was isolated by phenol extraction of SDS-lysed and proteinase K–treated peripheral blood leukocytes, and then amplified by the PCR procedure using an automated PCR thermal cycler (PerkinElmer Cetus, Norwalk, CT). The primers used for specific amplification of the polymorphic exon 2 domains of the DRB1, DQA1, DQB1, and DPB1 genes were previously described (27). Amplified DNA was digested by all-specific restriction endonucleases and subjected to electrophoresis using a 12% polyacrylamide gel. Digested fragments were detected by staining with ethidium bromide, and HLA genotypes were determined on the basis of the RFLP patterns generated as previously described (27).

Other. Ouchterlony double immunodiffusion was performed as described previously, using HeLa cell extract as antigen (10).

Cases. *Patient 1.* The patient, a 61-year-old Japanese woman, noticed chest pain, followed 3 months later by dyspnea on mild exertion. Chest radiography and computed tomography (CT) scanning showed bilateral basilar infiltrates. The patient had hypoxemia, with a restrictive pattern on pulmonary function tests. No muscle weakness was observed, and the creatine kinase (CK) level was normal (67 IU/liter). A lung biopsy specimen obtained by video-assisted thoracic surgery showed mild interstitial chronic inflammation and interstitial fibrosis lacking a temporal heterogeneity pattern, and a diagnosis of fibrotic nonspecific interstitial pneumonia was made.

Patient 2. The patient, a 51-year-old German woman, developed a nonproductive cough and dyspnea on exertion. Chest radiography showed bibasilar interstitial fibrosis, and pulmonary function tests showed a restrictive pattern with decreased diffusing capacity for carbon monoxide (DLco). A diagnosis of ILD was made, and the patient's pulmonary function remained stable throughout her disease course. She had polyarthralgia and developed erythema and keratosis of the palms and fingers consistent with mechanic's hands, but no cutaneous scleroderma, Raynaud's phenomenon, or DM rash (Gottron's papules or heliotrope rash) was observed. No muscle weakness was found, and the CK level was normal (56 IU/liter at the first visit) each time it was measured. When the patient was age 58 years, ovarian carcinoma was found, and surgery with subsequent irradiation was performed. She died of metastatic ovarian carcinoma at age 63 years.

Patient 3. The patient, a 72-year-old American woman, developed an itchy red eczematous rash that was thought to be due to a medication for hypertension. The rash was soon accompanied by progressive weakness, myalgias, mild dyspnea, and difficulty swallowing. She was started on prednisone and methotrexate, and 6 months after the rash had first appeared, she was referred to the Arthritis and Rheumatism Branch of the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health. There was a widespread maculopapular rash of the trunk, extremities, and head, and Gottron's papules were observed. Proximal muscle weakness was present, and her CK level was 358 IU/liter. Magnetic resonance imaging of the thighs showed both atrophy and probable inflammation on the STIR images. A biopsy of the deltoid muscle showed changes of an active inflammatory myopathy. No malignancy was identified. She was treated with pulse methylprednisolone. However, her muscle weakness and rash were not significantly improved, and infectious complications limited the therapeutic options. Her disease course was subsequently complicated by herpes zoster and the Ramsay-Hunt syndrome as well as by skin infections and cellulitis, mastoiditis, heart failure, and a cerebrovascular accident.

Patient 4. The patient, a 53-year-old Korean woman with intermittent episodes of productive cough due to bronchiectasis, noticed easy fatigability and myalgia in 1994 and later developed muscle weakness and was admitted to Seoul National University Hospital in February 1995. Proximal muscle weakness in her extremities and a dark pigmentation over the extensor surface of both knees were observed. The CK level was elevated at 3,808 IU/liter. The findings on electromyogram and muscle biopsy were consistent with inflammatory myopathy. A diagnosis of DM associated with ILD was made, and she was treated with prednisolone (60 mg/day). Her muscle enzyme levels gradually normalized, and her muscle weakness improved. Her chest radiograph and high-resolution

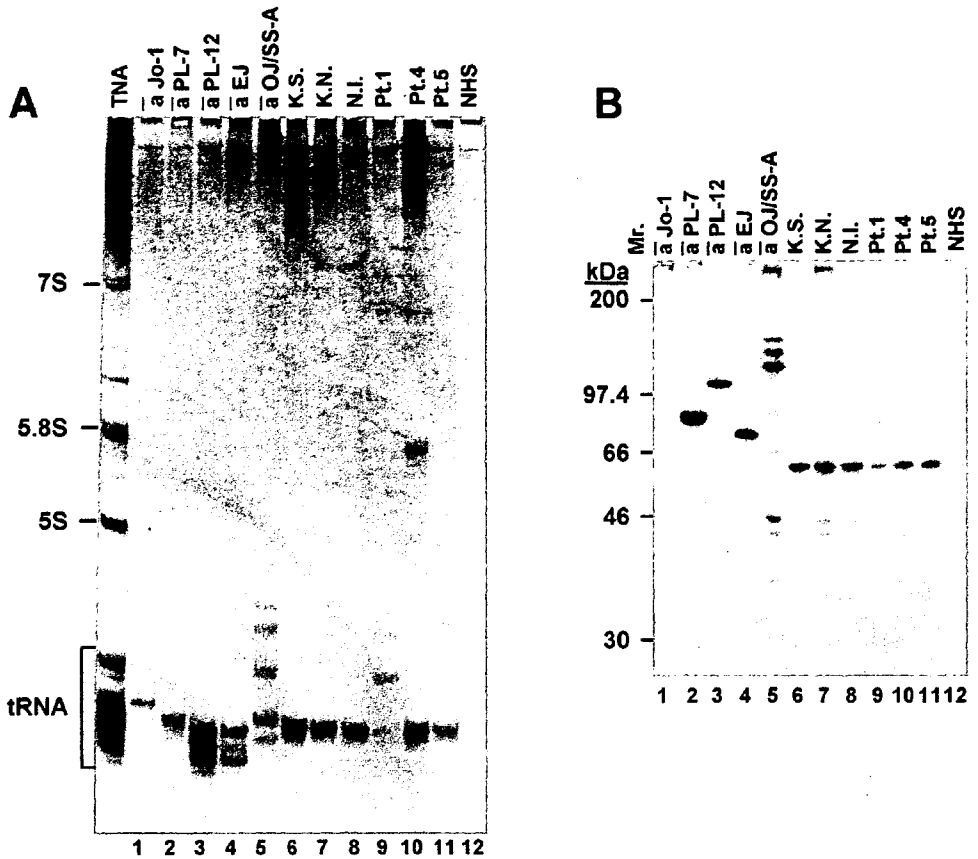

Figure 1. **A,** Immunoprecipitation (IP) for nucleic acids with anti-KS and control sera. Shown are patterns of transfer RNA (tRNA) resulting from 7*M* urea–10% polyacrylamide gel electrophoresis (PAGE) of phenol-extracted immunoprecipitates from HeLa cell extract, developed with silver stain. TNA = total nucleic acids, with the 5.8S and 5S small ribosomal RNAs and the tRNA region indicated. Antisynthetase sera used for IP are indicated. Lane 1, Anti–histidyl–tRNA synthetase (a Jo-1); lane 2, anti–threonyl–tRNA synthetase (a PL-7); lane 3, anti–alanyl–tRNA synthetase (a PL-12); lane 4, anti–glycyl–tRNA synthetase (a EJ); lane 5, anti–isoleucyl–tRNA synthetase (a OJ/SS-A); lanes 6–11, anti-KS sera from patients KS, KN, and NI in the previous study (23) and from patients 1, 4, and 5 in the present study; lane 12, normal human serum (NHS) control. The tRNA pattern with anti-KS sera is easily distinguishable from that of other antisynthetases. **B,** IP for proteins with anti-KS and control sera. Autoradiogram of 10% sodium dodecyl sulfate–PAGE of immunoprecipitates from ^{35}S-methionine–labeled HeLa cell extract. Mr. = molecular weight markers. Antisynthetase sera used for IP are indicated as in **A.** Anti-KS sera immunoprecipitated a very strong protein band from ^{35}S-methionine–labeled HeLa cell extracts (lanes 6–11), migrating at 65 kd, that was clearly different from the bands immunoprecipitated by sera with the described antisynthetases.

CT scan showed bilateral basilar interstitial fibrosis, and pulmonary function tests showed a restrictive pattern with decreased DLco. Her muscle weakness gradually improved, and the CK level normalized in January 1996. Prednisolone was tapered and discontinued in March 1996.

Patient 5. The patient, a 64-year-old Japanese man with a previous history of prostatic carcinoma, was admitted to the hospital due to bilateral infiltrates on chest radiography. He did not notice cough or dyspnea at that time, but a chest CT scan revealed bibasilar interstitial fibrosis. A transbronchial lung biopsy was performed, with histology showing usual interstitial pneumonia. He was started on prednisolone (40 mg/day), resulting in slight improvement seen on his chest radiograph. Prednisolone was tapered and discontinued in April 1998. He then developed polyarthritis and was treated with a nonsteroidal antiinflammatory drug. No muscle weakness was found, and the CK level was normal (50 IU/liter at the first visit) throughout his disease course.

RESULTS

Identification of anti-KS (anti-AsnRS) antibodies. Sera from all 8 patients (the 3 patients with ILD and/or inflammatory arthritis without evidence of myositis in our previous study [patients KS, KN, and NI; see

Table 1. Clinical features of 8 patients with anti-KS antibodies*

	Patient							
	KS	KN	NI	1	2	3	4	5
Age at onset, years/sex	36/F	44/F	61/F	60/F	51/F	72/F	53/F	65/M
Ethnic background	Japanese	Japanese	Japanese	Japanese	German	US	Korean	Japanese
ILD	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Myositis	No	No	No	No	No	Yes	Yes	No
DM rash	No	No	No	No	No	Yes	Yes	No
Arthritis	Yes	No	No	No	Yes	Yes	No	Yes
Malignancy	No	No	No	No	Ovarian cancer	No	No	Prostate cancer
Raynaud's phenomenon	No	Yes	No	No	No	No	No	No
Other autoantibodies	No	No	No	Anti-SSA/Ro	No	No	No	No
Diagnosis	ILD with arthritis	Idiopathic ILD	Idiopathic ILD	Idiopathic ILD	Idiopathic ILD	DM	DM	ILD with arthritis

* ILD = interstitial lung disease; DM = dermatomyositis.

ref. 23] and the 5 additional patients described above) were shown to immunoprecipitate a characteristic, identical pattern of tRNA, with a strong predominant nucleic acid band of tRNA size, accompanied by a faster faint band (Figure 1A). This gel pattern of tRNA was clearly distinguishable from the pattern of tRNA precipitated by the 5 other described antisynthetases (Figure 1A) and was identical in mobility and appearance to that of serum KS, the originally reported anti-KS serum (23) (Figure 1A).

A very strong band from ^{35}S-methionine–labeled HeLa cell extracts (Figure 1B), migrating at 65 kd, that was also identical in mobility to that of serum KS, was found by IP for all 8 sera, with 5 representative sera shown in Figure 1B. This was clearly different from the characteristic bands immunoprecipitated by sera with the other described antisynthetases (Figure 1B).

Five of the newly recognized anti-KS antibody–positive sera were tested for their ability to inhibit the in vitro enzymatic function of AsnRS (aminoacylation of $tRNA^{Asn}$). Four of the 5 new anti-KS sera significantly inhibited (by >50% at 10 minutes) AsnRS activity compared with normal serum or other controls (serum from patient KS by 87%, serum from patient KN by 99%, serum from patient NI by 91%, serum from patient 1 by 82%, serum from patient 2 by 100%, serum from patient 3 by 18%, serum from patient 4 by 87%, and serum from patient 5 by 91%). This inhibition was strong and comparable with that seen with serum KS, for 4 of the 5 new anti-KS sera. Purified IgG from the third new serum (from patient 3) showed significant, but not strong, inhibition (52%) that increased at 20 minutes (to 84%).

There was no significant inhibition of other synthetases. Normal control serum and anti-KS–negative myositis serum did not show significant inhibition of AsnRS, although sera with other antisynthetases inhibited the expected enzymes. These results indicated that sera with anti-KS by IP showed specific inhibition of AsnRS, further supporting previous data indicating that anti-KS reacted with AsnRS.

Clinical findings. The clinical features of the 5 newly identified patients (patients 1–5) and the 3 patients with anti-AsnRS reported previously (patients KS, KN, and NI) (23) are summarized in Table 1. All patients with anti-AsnRS antibodies were middle-aged or elderly, and 7 of them were women. Five patients were Japanese, 1 was from the US, 1 was German, and 1 was Korean. Seven of these 8 patients (88%) had ILD, documented in each case by both chest radiography and pulmonary function tests. In addition, 2 patients had myositis and a diagnosis of DM. Their clinical courses of ILD were classified as the chronic type. Four patients (50%) had nonerosive arthritis or arthralgia. Raynaud's phenomenon was seen in only 1 patient. None of the patients had sclerodactyly or overlap syndromes with other connective tissue diseases. Malignant diseases (ovarian carcinoma and prostatic carcinoma) were observed in 2 patients. Regarding other autoantibodies, anti-SSA/Ro antibodies were detected in only 1 patient.

Anti-AsnRS was found in 0% of Japanese patients with myositis, but was found in 3% of Japanese patients with "idiopathic" ILD. Thus, most patients with anti-AsnRS antibodies had chronic ILD with or without features of PM/DM or other connective tissue disease.

Immunogenetic features. The HLA class II gene was determined in 4 Japanese patients (Table 2). All 4 patients had DR2 (DRB1*1501 or DRB1*1502) compared with 33% of healthy local controls. It should be noted that all patients with anti-AsnRS antibodies had DR2, but the frequency of DR2 did not reach statistical significance ($P > 0.05$).

Table 2. HLA class II genes in Japanese patients with anti-KS autoantibodies

	Patient			
	KS	KN	NI	1
DR	2/5	2/1	2/2	2/4
DRB1*	1502/1101	1501/0101	1502/1502	1501/0405
DQA1*	0103/0501	0102/0101	0103/0103	0102/0303
DQB1*	0601/0301	0602/0501	0601/0601	0602/0401
DPB1*	0901/1401	0201/0501	0901/0901	0201/0402

DISCUSSION

We have identified anti-KS (anti-AsnRS) autoantibodies in 8 patients with ILD and DM, by IP of the same distinctive set of tRNA and protein that differed from those precipitated by the other 5 antisynthetases. Most of the anti-KS sera showed specific inhibition of the enzyme target, AsnRS, without inhibiting other synthetases.

Several interesting characteristics of the previously studied antisynthetases have been described: 1) they are associated with a distinctive clinical syndrome referred to as the antisynthetase syndrome, 2) they are directed at functionally related enzymes (performing the same function for different amino acids), 3) they do not cross-react with other synthetases, and 4) they tend to be mutually exclusive. Anti-AsnRS antibodies seem to have the same features. No serum with any other antisynthetase has had antibodies to AsnRS, and none of the 8 anti-AsnRS sera reported here showed signs of reaction with other synthetases. The mechanism of this phenomenon remains unknown.

Multiple tRNA bands immunoprecipitated by anti-AsnRS were found on urea-PAGE. The patterns of tRNA for each of the 8 patients were very similar, highly restricted compared with total tRNA, and distinctive compared with the pattern of other anti–aminoacyl tRNA synthetase autoantibodies. These bands are likely to represent different forms of tRNA for asparagine, which can include tRNA with different asparagine anticodons (uracil-uracil-adenine, uracil-uracil-guanine) or tRNA with the same anticodon but differences in other parts of the sequence. Most sera with anti-HisRS, anti-ThrRS, anti-GlyRS, and anti-IleRS had not been described to react directly with tRNA, suggesting indirect precipitation of tRNA. However, approximately one-third of anti-HisRS–positive sera were reported to contain autoantibodies recognizing $tRNA^{His}$ (28). Most anti-AlaRS sera react directly with the sets of $tRNA^{Ala}$ with the inosine-guanine-cytosine anticodon (29). We previously found that the 3 original anti-KS (anti-AsnRS) sera did not immunoprecipitate any RNA from deproteinized HeLa extracts (23). This suggests that anti-AsnRS antibodies can precipitate $tRNA^{Asn}$ indirectly, through its affinity for AsnRS, although the possibility of conformational epitopes on the tRNA has not been excluded (28). Further analysis will be necessary to determine the sequence and specificity of tRNA immunoprecipitated by anti-AsnRS.

The specific inhibition of AsnRS function by most of the sera found to have anti-KS is consistent with findings observed for other antisynthetases. It should be noted that our anti-KS sera also demonstrated inhibition of enzymatically active recombinant AsnRS (30). Most sera with any of the 5 reported antisynthetases specifically inhibit the aminoacylation of the respective tRNA, indicating inhibition of the enzymatic function of the synthetase (3,5–7,12). This functional inhibition may indicate that the autoantibodies are recognizing the active sites of the synthetases. In contrast, it has been reported that animal antisera raised against synthetases do not consistently show such inhibition, suggesting that active sites tend not to be immunogenic for animals (31). Hypothetically, this could relate to relative conservation of the active site. However, there might be an alternative mechanism for inhibition. For example, binding of antibodies outside the active site may alter the structure of the enzyme or interfere with enzyme activity sterically. Further studies of the precise epitope on the aminoacyl–tRNA synthetase might help to explain the development of these autoantibodies.

Each of the 5 previous antisynthetases was first identified in patients with myositis and then found to be associated with ILD. In previous studies, these autoantibodies were associated with myositis with a high frequency of ILD (50–80%) and arthritis (50–90%) (1,2,17,18), as well as an increase in Raynaud's phenomenon (60%), fever with exacerbations (80%), and the skin lesion of the fingers referred to as mechanic's hands (70%) when compared with the overall population of patients with myositis (9–11). The similarities between patients with different antisynthetases have been noted, whereas certain differences have been found, which must be considered preliminary due to the small reported number of patients with non-HisRS antisynthetases. Absence of significant myositis over the full disease course in patients with anti-HisRS is rare (<5%) (32), whereas patients with anti-AlaRS are more likely than patients with anti-HisRS to have ILD and/or arthritis without clinical evidence of myositis (19). Anti-ThrRS

resembles anti-HisRS more than anti-AlaRS in Japanese patients (33).

In the present study, 7 of 8 patients (88%) with anti-AsnRS autoantibodies had ILD, some with other associated features of connective tissue disease including arthritis and Raynaud's phenomenon. In this respect, anti-AsnRS appears to resemble anti-AlaRS more than anti-HisRS. It is noteworthy that the 2 patients with both anti-AsnRS and myositis were among the 3 patients from outside Japan, while none of 5 patients from Japan had myositis. Thus, as with patients with anti-AlaRS, for patients with anti-AsnRS, the frequency of ILD without myositis may be higher in Japanese patients. However, most of the group of patients with ILD without myositis who were tested in this study were from Japan.

The features of these 8 patients with anti-KS appeared to reside within the spectrum of the antisynthetase syndrome that has been associated with other antisynthetases. ILD is one of the most important features of the antisynthetase syndrome, and Raynaud's phenomenon and arthritis, as seen in some patients with anti-AsnRS, are also likely to be part of the syndrome. The syndrome associated with anti-AsnRS may be one end of the spectrum of patients with antisynthetase. This highlights the clinical importance of looking for such antibodies in patients with ILD even if there are no signs of myositis or connective tissue diseases.

The typical cutaneous features of DM were observed in 2 patients with anti-AsnRS antibodies. PM has been reported to be much more common (60–80% or more) than DM in patients with anti-HisRS in most studies, whereas DM was most frequent with anti-GlyRS (15) and was also found to be common among patients with anti-AlaRS (13). Like anti-GlyRS and anti-AlaRS antibodies, anti-AsnRS antibodies were more associated with DM in the small number of patients available.

Malignancy has been reported to be unusual in patients with antisynthetases. In our studies, 2 patients were found to have malignancy during their disease course. However, malignancy in these patients may not be related to the DM or ILD, since these malignancies occurred separated in time from each other.

Immunogenetic studies of connective tissue disease have been performed, but HLA associations produced conflicting results. However, a strong correlation of HLA class II antigens with some autoantibodies has been reported (34). With regard to antisynthetase antibodies, HLA–DR3 (DRB1*0301), DQA1*0501, or DQA1*0401 was found to be significantly increased in myositis patients with antisynthetases (9,21). In Japanese patients, we have reported that 7 of 9 patients (78%) with anti-HisRS tested had the HLA class II DRB1*0405;DQA1*0302;DQB1*0401 haplotype, compared with 22% of healthy controls (odds ratio [OR] 13, $P = 0.002$), while 4 of 7 patients (57%) with anti-AlaRS had the DRB1*1501;DQA1*0102;DQB1*0602 haplotype, compared with 9% of healthy controls (OR 14, $P = 0.006$) (35). Interestingly, all 4 Japanese patients tested had DR2 (DRB1*1501/1502), compared with 33% of healthy controls, although a definite statistical association could not be established. These results suggest that the stronger association of anti-AlaRS and anti-KS with ILD may be related to the DR2 phenotype. However, it has been noted that different ethnic groups exhibit different immunogenetic profiles that link with specific autoantibodies (36). Therefore, further studies including analysis of more patients with anti-KS antibodies in different ethnic groups and major histocompatibility complex–restricted T cell responses could provide important clues for understanding the possible mechanisms for the development of antisynthetase antibodies.

The mechanism for the association of antisynthetases with ILD is unknown, but it seems to be related to etiologic factors (37). Recently, a new association of anti-HisRS–positive PM and ILD was reported in a patient with hepatitis C virus infection (38). It was hypothesized that viruses might interact with the synthetases and induce autoantibodies by molecular mimicry or antiidiotype mechanisms in the anti-HisRS–positive patient with myositis associated with ILD (3,39). Another mechanism for generating autoantigenic epitopes of synthetase by granzyme B cleavage in apoptosis was also described recently (40,41). However, these proposed mechanisms remain speculative, and further studies could provide important clues for understanding the possible mechanisms for the development of these antibodies. Studies of these antibodies may provide insight into the etiologic and pathogenetic mechanisms of ILD and myositis.

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AUTHOR CONTRIBUTIONS

Dr. Hirakata had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Hirakata.
Acquisition of data. Hirakata, Nagai, Genth, Song, Targoff.
Analysis and interpretation of data. Hirakata, Suwa, Takada, Sato, Mimori.
Manuscript preparation. Hirakata, Takada, Targoff.
Statistical analysis. Hirakata, Suwa, Targoff.

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関節リウマチの治療

メトトレキサートの使い方と注意すべき副作用

鈴木康夫* 若林孝幸 齋藤榮子 諏訪 昭**
東海大学医学部内科学系リウマチ内科学 *教授 **助教授

SUMMARY
・メトトレキサート(MTX)はRA治療のanchor drugと位置づけられる.
・活動性が高く,RF陽性,骨びらんのあるような予後不良例では早期より使用すべき薬剤である.
・週6mgより開始し効果不十分であれば4～6週後に増量する.
・週8mgあるいは0.2mg/kg体重以上使用するとき葉酸週3～5mgを併用する.
・注意すべき副作用は骨髄障害,間質性肺炎,感染症である.禁忌・慎重投与例を見きわめ,適切なモニタリング,発生時の初動処置が重要である.

はじめに

メトトレキサート(MTX)は高い有効率,継続率と優れた骨破壊進行抑制効果,生活機能改善効果にくわえ生命予後の改善や心筋梗塞の発症率減少効果を兼ね備えた唯一の抗リウマチ薬(DMARD)である.海外ではRA治療のanchor drugに位置づけられている.わが国でも使用量は年々増加し,推定14～5万人のRA患者に使用されている.しかし,投与量の上限が週8mgであるにもかかわらず致死的副作用の報告が増加している.MTXがわが国においてRA薬物療法のanchor drugになるには,用量,葉酸投与法,安全性管理などの問題点が残されている.本稿では,MTXのRAに対する基本的投与法,葉酸の使い方および注意すべき副作用とそのモニタリング・対処法を概説する.

Ⅰ.投与法(表1)

① 開始時投与量

欧米ではMTX7.5mg/週から開始し4～8週後に効果により,10～15mgへ増量,さらに効果不十分であれば20～25mgへ増量する方法が一般的である.しかし,7.5mg/週で治療を開始した場合,6週後に効果不十分で増量が必要となる症例が66～97%であることから,開始時投与量は10mg未満であるべきでないとの勧告もある[1].わが国では添付文書上の制限もあり,8mg/週以下でスタートする.東海大70例のMTXの有効性と安全性を70例で前向きに検討した結果,1年後の継続例は58例で,最終投与量の内訳は週6mg:6例,8mg:21例,10mg:21例,12～12.5mg:8例,15mg:2例であった.週4mgでの有効例がなかったことから週6mgからの治療開始が適当と思われる.しかし,高齢(>70歳),低体重(<40kg),腎機能低下などリスクがある例では低用量で開始する.

表1　MTXの基本的投与法

①開始投与量	週6mgがstarting doseとして推奨される 例外）以下の危険因子：低体重（＜40kg），高齢（＞70），腎機能異常（s-Cr＞基準値）
②最大投与量	最大週15mg．保険上の適用用量は週8mgを越える際は，説明と同意が必要 6mg/週で投与開始後4～6週目で効果不十分であれば8mg/週に増量 効果の減弱（エスケープ現象）の際は，週2mg増量 危険因子のある例は週8mgまでを上限とする
③葉酸の投与	全例に葉酸併用は必要でない 予防的併用：週3～5mg，MTX最終投与から36～48時間後内服 投与量＞8mg/週全例，高齢者，腎機能異常（s-Cr＞基準値） 葉酸の潜在的欠乏（MCV＞110） 治療的併用：週5mg，MTX最終投与から36～48時間後内服 肝酵素上昇（AST，ALT＞50IU/L，2回連続あるいは＞基準値×3） 口内炎，消化管症状（嘔気，下痢） 白血球減少＜4000/μL，血小板＜100,000/μL，大球性貧血（Hb＜10g/dL） ロイコボリン救命投与：白血球減少＜2,000/μL，血小板＜50,000/μL，MTX関連リンパ増殖性疾患，重篤な感染症，重篤な肝障害
④投与開始時チェック項目	末梢血（MCV，白血球分画），肝機能（Alb含む），腎機能，KL-6，胸部XP（正側面）， HBV，HCV（肝酵素＞基準値，輸血歴があれば），免疫グロブリン 胸部XP異常所見，KL-6高値があれば胸部CT，SaO_2
⑤副作用モニタリング	末梢血（MCV，白血球分画），肝機能（Alb含む），腎機能，以上1～2ヵ月ごと，IgG（3ヵ月ごと）， 胸部XP：6ヵ月から1年ごと

② 最大投与量

MTXの投与量別効果をDB-RCTで検討した成績では5～15mg/週の間で用量依存性が示されているが，週15mgを超えると，一部の治療効果の指標はプラトーに達してくる[2]．最近のdose escalation studyでは週15mg経口投与されている症例を筋注で週45mgまで増量しても，有効率の増加はなかった[3]．この結果は，RAに対するMTXの効果は週15～20mgでほぼ最大に達することを意味している．

厚生労働省研究班でMTX（8mg/週＋葉酸5mg/週），BUC（200mg/日），両者同時併用療法の有効性と安全性を検討した多施設協同二重盲験試験ではACR20，50 responseはそれぞれ43.5%，34.8%とブシラミン単剤と同等であった[4]．保険適用用量範囲内でのMTXの有効性は海外の成績に比べて，はるかに低い有効率であり，葉酸を併用すればさらに有効率が下がる可能性が大きいことを示唆する．

東海大学の検討では週8mgまでの投与量でのACR20，50，70 responseは61.4%，35.1%，12.3%であるが，最高週15～20mgまで投与すれば84.2%，64.9%，35.1%と海外データと同程度の高い有効率が得られる．また，MTX投与中に治療効果の減弱（エスケープ現象）をきたし，週8mgで対応できなくなった症例が5.3%あったが，増量して週10mg以上使用すれば効果が再現された[5]．また，東海大58例の1年後のX線上の骨・軟骨破壊進行を検討した結果，MTXの平均投与量は9.35mg/週（6～15mg/週）で，骨びらんスコアが進行しなかった例が50%みられた．DAS＜2.6以下の寛解例に進行例はなく，ACR 70 response，DAS good responseを満たした症例では非進行例が多かった．一方，厚生労働省研究班の臨床試験では，MTXとBUCの骨破壊進行抑制効果は同等であった．保険適用内用量のMTXの関節破壊進行抑制効果は他のstrong DMARDsと同程度であるが，週8mgを超えて使用すれば，関節破壊進行阻止ができることを示唆する．骨・軟骨破壊

進行の抑制・阻止が達成できる著効例や寛解例が週8mgを超えることにより増加することは重要な点である．東海大の検討ではMTX週15mgを超えて必要な症例は非常に少なく，わが国のRA患者の平均体重が米国より15kg少ないことを考えれば，最大投与量は15mg前後が適当であろう．

③ 投与法

わが国では投与間隔は12時間ごとに1～2日にかけて分割投与することが多いが分1投与でも有効性，安全性に差はない．筆者は服薬コンプライアンスを考え，分1～2で週1日投与としている．

④ 第一選択薬として使用されるべき例

MTXもほかのDMARDsと同様に早期に使用するほど有効率，継続率，骨破壊進行抑制効果が優れ，エスケープ現象も少ない．比較的効果発現も速いので活動性が高く(DAS＞5.1)，予後不良因子(RF陽性，抗CCP抗体陽性，既骨びらん)のある例ではなるべく早期から使用する．とくに，若年齢(＜50歳)の症例は副作用頻度も少なく第一選択薬として使用すべきである．

⑤ 葉酸の使用法(表1)[6]

a．適　応

葉酸併用投与により肝酵素上昇，血球減少症，口内炎，消化器症状など用量依存的副作用の予防・治療が可能である．米国では葉酸1～2mg/日の併用はRAに対する治療効果に影響しないため，MTX投与の全例に使用すべきとの立場である．筆者は投与量の少ないわが国では全例には必要がないとの立場である．その根拠は①葉酸併用により治療効果が減弱する症例がみられ，②MTX0.15mg/kg/週以下では用量依存性副作用頻度が少ない，③これらの副作用は発現後に葉酸を併用してもほとんど改善する，である．葉酸予防投与の適応としては，用量依存的副作用の頻度が高くなるMTX0.2mg/kg体重以上の投与例，70歳以上の高齢者，腎機能低下例があげられる．

b．葉酸投与量

筆者の検討では葉酸5mg/週の併用により約半数に治療効果の減弱がみられたが葉酸3mg/週では治療効果の減弱は30%以下で，副作用改善率変わらなかった．しかし，肝酵素の改善は上昇に対しては，週5mgのほうが改善が速かった．葉酸/MTX比＝0.3～0.5：1の葉酸投与量で用量依存的副作用の大半は改善できることから，副作用の予防目的では葉酸3mg，治療目的では5mg/週が適当と考える．葉酸製剤とMTXの投与間隔については明確な結論はでていないが，フォリアミン®3～5mgをMTX最終服用後24～48時間あけて投与すれば効果に大きな差はない．フォリアミン®の併用により副作用が改善しても，臨床効果が減弱した場合は，筆者は葉酸を減量している．週2～4mgの間で調節すれば，副作用の再発なくRAに対する効果も持続する場合が多い(図1)．

⑥ 併用療法の基本薬として

DAMRDs併用療法を欧米では単剤より優れていると位置づけている．DB-RCTで有効性が確認できた併用療法の組合せは数少ないが，治療効果が明かな組み合わせを使用すべきである．わが国では厚生労働省研究班の調査研究によりMTX＋ブシラミン同時併用療法が単剤に比べて有効率，骨破壊進行抑制効果において優れていることが示されている．

一方，欧米では生物学的製剤とMTXの併用が積極的に行われている．早期RAに対する生物学的製剤とMTXとの併用(infliximab：ASPIRE試験，etanercept：TEMPO試験，adalimumab：PREMIER試験，tocilizumab：CHARISMA 試