表1 主な遺伝子多型の種類とその特徴[1]

	RFLP	VNTR	マイクロサテライト	SNP
必要なサンプル量	数μg	数μg	10ng	10ng
多様性	+	++	++	+
判定法	サザンブロット法	サザンブロット法	PCR	PCR
判定の容易さ*	2	3	4	1
ゲノム上に存在するコピー数	数万	数千	数万	300万-1,000万

*容易な順に1-4，RFLP: restriction fragment length polymorphism，VNTR: variable number of tandem repeat，SNP: single nucleotide polymorphism.

これが一定以上の頻度(遺伝学的には，通常，人口の1%以上の頻度で存在するものと定義されており，1%未満のものはバリエーションと呼ばれている)で存在することをいう.

遺伝子多型の種類は下記のように分類される(**表1**)[1].

(1) 1から数十塩基(数千塩基のこともある)が欠損や挿入をしているもの(挿入/欠損型).

(2) 1個の塩基が他の塩基に置き換わっているもの(single nucleotide polymorphism: SNP).

(3) 2塩基から数十塩基を1単位とする配列が繰り返し存在する部位において，その繰り返し回数が個人間で異なるもの.

(2)のSNPは数百塩基対から1,000塩基対に1カ所程度の割合で存在し，ゲノムの中には300万-1,000万のSNPが存在すると考えられる.また，DNAの特定の制限酵素認識配列にSNPがあるものをRFLP(restriction fragment length polymorphism: 制限酵素切断断片長多型)と呼ぶ.

更に，(3)の繰り返し単位が2-4塩基のものをマイクロサテライト多型，数塩基から数十塩基のものをVNTR(variable number of tandem repeat)という.

これらの特徴については**表1**を参照されたい[1].

II. 感染症と関連が検討されている遺伝子多型

既に，サイトカインなど感染に関与する多数の遺伝子において，各々多数の遺伝子多型が報告され，**表2**のようなサイトカイン，(可溶性)受容体分子，toll like receptor(TLR)，NFκB，plasminogen-activator-inhibitor-1(PAI-1)，heat shock protein(HSP)などの遺伝子多型において種々の感染症や敗血症との関連が検討されつつある.

本稿では，SIRSや感染に関連する主なメディエータの遺伝子多型について解説する.

III. サイトカイン

1. Tumor necrosis factor(TNF)

TNF遺伝子の多型と感染症の関連に関してはサイトカインの中で最も多くの研究が行われてきた.

TNFの遺伝子のpromoterの−308位にある多型のように，−308位のG(TNF1)がAに変化するSNP(TNF2)では，DNAからmRNAへの転写活性が高いことが報告されている[2,3].また，57例の健常人の献血ドナーの遺伝子多型解析によって，全血のLPSによる刺激でのTNF産生量は16例のTNF1/TNF2の患者で，41例のTNF1 homozygotes(TNF1/TNF1)の患者よりも高いことが示されている[4].

しかしながら，TNFをはじめ種々のサイトカイン遺伝子多型とその産生量の関連に関しては，**表3**のように結果は必ずしも一定しないことが多い.

一方，TNF遺伝子多型と感染の罹患しやすさや予後に関しても多数の研究が行われてきた(**表4**).

−308位のGがAに変化した多型では，敗血症性ショックで死亡率が3.7倍となるという報告があり[5]，また，手術や外傷患者でもそのよう

表2 敗血症に関連するものとして検討されている遺伝子多型

TNFα: −376位(G/A), −308位(G/A)(TNF1/2), −238位(G/A), *Nco*Iによる RFLP(TNFB1/2), CG repeats
TNFβ: +250位(G/A)
TNFγ: +874位(T/A)
IL-1: −511位(C/T), −31位(C/T), 3953位(C/T)
IL-6: −597位, −572位, −373位, −174位(G/C)
IL-10: −1082位(G/A), −819位(C/T), −592位(C/A)
IL-18: −607位(C/A), −137位(G/C)
IFNγR1: 88位(G/A), R2: 839位(A/G)
IL-1ra: 86 bpの VNTR, +2018位
PAI-1: 4G/5G
CD14: −260位(C/T), −159位(C/T)
TLR2: Arg753Gln
TLR4: Asp299Gly, Thr399Ile, CA repeats(A1-18)
Fcγ receptor III, CD16(Fcγ receptor IIIb): NA1/NA2
CD32(Fcγ receptor IIa): H/H 131-R/R 131
ICAM-1: Gly241ArgR
HSP-70: *Nco*Iによる HOM(C/T), *Pst*Iによる G/A
Caspase12: Csp 12-Sと Csp 12-L

PAI-1: plasminogen-activator-inhibitor-1, TLR: toll like receptor, HSP: heat shock protein.

な傾向は示されている[6,7]. しかし, 腹部に由来する敗血症とは相関がないという報告もある[8].

市中肺炎患者での敗血症性ショックの罹患率では−308位の遺伝子多型との関係はないが, lymphotoxin α (TNFβ)(LTα)遺伝子の+250位がAAの場合, TNFの産生量が増加し, LTα +250位がAAの場合には敗血症性ショックや敗血症性ショックによる死亡率が高いことが報告されている. しかし, 反対のGGの場合にはショックを伴わない呼吸不全が多いと報告されている[8].

このLTα遺伝子はTNF遺伝子の近傍に存在し, LTα +250位とTNF −308位の両者の間にlinkage disequilibrium(連鎖不均衡)が認められ[9], また, LTα遺伝子と heat shock protein 70 (HSP-70)遺伝子との間にも連鎖不均衡が認められている[10].

連鎖不均衡とは2つ(以上)の遺伝子の変異が連関して存在していて, Mendelの独立法則に従わないもので, ある遺伝子多型と他の遺伝子多型が連関して遺伝してしまうことである. 同じ染色体でアレル間の距離が短いほど, また, 新しいものであるほど連鎖不均衡が強いといわれる. このため, ある遺伝子多型がある疾患と相関しているようにみえても, 実際には, その近傍の連鎖不均衡の他の遺伝子多型が疾患と関連している可能性は否定できない. 特に, TNF遺伝子が存在する第6染色体には, 近傍に他の免疫に関連する遺伝子が多数存在する. そのため, 上記のTNF −308位, LTα +250位の炎症反応との関連も実は, これらと連鎖不均衡である未知の遺伝子多型が関連しているのかもしれない.

このような点から, 疾患とある遺伝子多型が直接関連していることの証明は難しく, その判断は慎重にする必要がある.

2. Interleukin-1(IL-1)

IL-1 familyとしては, IL-1A, IL-1B, およびレセプター拮抗作用のあるIL-1 receptor antagonist(IL-1ra)の存在が知られている.

Readらによると, IL-1B(−511位)とIL-1ra (+2018位)を1,106例の髄膜炎症例で検討したところ, 死亡には, 年齢, 起炎菌の種類, 両者の遺伝子多型が独立して関連していたと報告し

表3 TNF遺伝子座の多型によるTNFαまたはβ産生量の変化

遺伝子多型	TNFαの発現	文 献
−1031	increased	[Higuchi, 1998]
	no difference	[Kaijzel, 2001]
−863 A	increased	[Hohjoh, 2001]
	decreased	[Skoog, 1999]
−862(*−863)	increased	[Higuchi, 1998]
	no effect	[Uglialoro, 1998] [Kaijzel, 2001]
−857 T	increased	[Hohjoh, 2001]
	no difference	[Kaijzel, 2001]
−856(*−857)	increased	[Higuchi, 1998]
	no effect	[Uglialoro, 1998] [Kaijzel, 2001]
−574	no effect	[Uglialoro, 1998]
−376 G/A	no effect	[Huizinga, 1997] [Kaijzel, 1998] [Bayley, 2001]
−308 G/A	no effect	[Bayley, 2001] [Pociot, 1993] [Turner, 1995] [Huizinga, 1997] [Uglialoro, 1998] [Sotgiu, 1999] [Kaijzel, 2001]
(A: TNF2)	increased	[Huang, 1999] [Kroeger, 2000] [Maurer, 1999] [Wilson, 1997] [Galbraith, 1998]
−244, −238	increased (in certain cell lines)	[Bayley, 2001]
−238 A	increased	[Grove, 1997]
	decreased	[Kaluza, 2000]
	no effect	[Pociot, 1995] [Huizinga, 1997] [Kaijzel, 1998] [Uglialoro, 1998]
+70 G/A	no effect	[Uglialoro, 1998]
+489	no difference	[Kaijzel, 2001]
TNFa, b, c, d, e microsatellite		
a	no effect (TNFβ)	[Pociot, 1993]
a2	decreased	[Derkx, 1995]
a2 and a9	increased	[Obayashi, 1999]
a13	decreased	[Obayashi, 1999]
c	no effect (TNFβ)	[Pociot, 1993]
d3	increased	[Turner, 1995]
LTα (TNFβ) Intron 1, *Nco*I RFLP (+250 G/A), (Thr26Asn) (TNFB*1=Asn26; TNFB*2=Thr26)		
	no effect (TNFβ)	[Pociot, 1993]
TNFB1 (Asn26)	increased	[Messer, 1991]
TNFB2 (Thr26)	decreased	[Messer, 1991]

各文献はhttp://www.bris.ac.uk/pathandmicro/services/GAI/cytokine4.htmを参照.
*著者による注.

ている[11].

IL-1raには2番目のintronに存在する，86 base pairのVNTRが存在し，repeatsの数により5種類に分かれ，最も短いA2遺伝子型では敗血症に陥りやすいという報告がある[12]. しかし，それらとの関連はないという報告もある[13,14].

3. Interleukin-10 (IL-10)

IL-10はanti-inflammatoryなサイトカインと考えられており，遺伝子多型も多数報告されている. 髄膜炎で死亡した症例の家族ではIL-10の産生量が多い，−1082位のSNPの頻度が高いなどの報告もみられる[15].

表4 敗血症と種々の遺伝子多型の関連

サイトカイン多型	疾患	関連(症例数)	文献
LTα intron 1, *Nco*I RFLP	外傷後重症敗血症	あり(n=110)	[Majetschak, 1999]
+250(A: TNFB2)	市中肺炎での septic shock の罹患率	あり(n=280) (RR: 2.48(1.28-4.78))	9)
	重症敗血症	あり(n=40)	8)
		あり(生存率は関連なし) (n=93)	12)
	敗血症の進行(新生児)	なし(n=23)	[Weitkamp, 2000]
TNFα −308(A: TNF2)	敗血症性ショック	あり(死亡率3.7倍(n=176))	5)
	重症敗血症	なし(n=223)	8)
	敗血症性ショックの死亡率	あり(n=112)	[Tang, 2000]
	外傷後の重症敗血症罹患率	あり(OR 4.6)(n=152)	6)
	外傷後の死亡率	あり(OR 2.1)(n=152)	6)
	市中肺炎での septic shock の罹患率	なし(n=280)	9)
TNFα −308(AA) or LTα +250(AA)	市中肺炎での septic shock の罹患率	あり(n=280) (RR: 2.51(1.30-4.87))	9)
IL-1ra intron 2, 86 bp VNTR(A2 allele)	重症敗血症	あり(n=354)	12)
IL-1b +3953 C/T(TaqI)	敗血症の罹患率や敗血症の予後	なし(n=354)	12)
IL-6 −174(G/G)	敗血症生存率	あり(n=326)	[Schluter, 2002]
PAI-1 promoter(4G/4G)	髄膜菌による敗血症の予後	あり(n=401)	17)
	外傷患者での敗血症罹患率や予後	なし(n=61)	18)
CD14 −159(T/T)	敗血症性ショック	あり独立危険因子(OR 5.3) (n=90)	19)
(C/T)	敗血症の発生率や死亡率	なし(n=451)	[Hubacek, 2000]
TLR4 Asp299Gly allele	敗血症性ショック	あり(n=164)	20)

LTα: lymphotoxin α, つまりTNFβの遺伝子, IL-1ra: IL-1 receptor antagonist, PAI-1: plasminogen activator inhibitor-1, OR: odds ratio, RR: relative risk, 95% CI: 95%信頼区間

−592位のSNP(C/A)は，刺激によるIL-10産生量や死亡率に関連するという報告もあれば[15]，いずれにも関連がないという報告もある[16]．

4. PAI-1

更には，PAI-1ではpromoterに存在する1塩基の有無で4Gまたは5Gと分類される多型があり，4G/4Gでは髄膜菌による敗血症の予後が悪化するという報告がある一方[17]，外傷患者での敗血症罹患率や予後には差がないという報告もある[18]．

IV. 抗原認識分子

1. CD14

エンドトキシンのレセプターであるCD14では，−159位のCからTへの多型で，soluble CD14が高値を示すなどの報告や，TT型は敗血症性ショック例に多く，多重ロジスチック解析で，TT型は独立危険因子であるという報告がある(odds ratio: 5.30, 95% CI: 1.20-22.50)[19]．

2. TLR

TLRには既に少なくとも14種類のものが報告されており，TLR2はグラム陽性菌や真菌などに，TLR4はグラム陰性菌に関与していると報告されている．TLR4の299番目のアミノ酸がAspからGlyへの多型では，敗血症性ショックやグラム陰性菌感染である頻度が高いことが示されている(**表4**)[20]．

V. 現状の問題点

前述のように種々のサイトカインの遺伝子多型によってサイトカインの産生量の変化が生じることが報告されているが，サイトカインの遺伝子多型とサイトカインの産生量や機能の変化との関連は報告によって一定していないことも多い(**表3**)．これは，細胞や，サイトカインを産生させる刺激やその時期によってサイトカイン産生量は大きく変化することも一因である[2]．更に，LPSに対するIL-6の産生量は，IL-6そのものの遺伝子多型よりも，性や，IL-6よりもサイトカインネットワークで上流にあるTNFαやIL-1βの反応性に左右される(TNFα，IL-1βの産生量が多いときにはIL-6が高い)ことが報告されている[21]．

また，サイトカイン産生量に直接関与しないpromoterやintronなどでの変異でも産生量に関連するという報告も多い．

これらの原因に，先の連鎖不均衡による他の遺伝子の多型が実際は関与している可能性もある．

既存の報告はほとんどが100例以下の小規模な研究で，既報にて指摘したように1例の変化で有意差が消失してしまうような報告や関連の有無が相反する報告も多数あり，現段階では結果の解釈には十分注意を要する必要がある[22]．

そのほか，人種間によって多型の頻度の差が存在し，場合によっては反応性にも差がある可能性もある．また，同一民族間さえ，対象が異なると未知の要因によって結果が異なるという報告もある．

そのため，日本人において大規模で，かつ，対象を絞った臨床研究が必要である．

VI. その他のSIRSや炎症に関連する遺伝子多型

ヒトの炎症に関連する多型のみではなく，細菌そのものにも遺伝子多型が存在し，それらの情報は，感染微生物の同定や集団発生時などの感染経路の疫学的調査に応用されている．

また，肝チトクロームp450(CYP)2C19などの薬剤代謝酵素の遺伝子多型は薬物の代謝速度に影響し，薬剤血中濃度がヒトによって数倍から数百倍異なり，副作用の原因や治療効果を示すことができない一因とも考えられている．

VII. 今後の展望

SIRSや炎症反応と遺伝子多型の罹患率や予後，あるいは，細菌，薬剤代謝などに関する情報が信頼に足るものとなれば，その情報は臨床において以下のように非常に有益なものとなる．

1. 適切な薬物投与方法が可能になる

前述のように薬物代謝に関連する遺伝子多型も多数判明してきており，患者ごとに治療薬やその投与量を変えることも可能となってくる．

これらの情報により，既存の治療法や種々の抗サイトカイン療法など，今までは患者の不均一性のために治療効果を示せなかったものが，遺伝子多型解析によって，侵襲に対する反応性や薬物動態のより均一で適切な患者群を対象にすることによって有効性を示すことができるかもしれない．

2. 罹患率や予後の予測，個々の患者ごとのより適切な医療が可能となる

以上のように，各々の患者ごとに遺伝子多型情報を得ることによって，個々の患者ごとの治療選択をより詳細に行うことができる．

遺伝子多型情報によって感染症に罹患しやすい患者，罹患した場合に重篤化や死亡など予後の悪い患者などが鑑別でき，感染症のリスク判定が可能となり，予防，発症の遅延，早期発見/治療が可能となる．

また，重症化しやすい患者には，あらかじめ手術などの侵襲が少ないものやより安全な治療法を選択したり，発症時にはより早期から集学

的な治療を行うことも可能となる．一方，重症化しにくい患者には一般病床での管理で済み，遺伝子多型情報により，人的，資金的な効率化が図られる可能性がある．

例えば，同じ侵襲でもサイトカインなどのメディエータを過剰に産生する患者では抗メディエータ療法を行ったり，過少にしか産生しない患者では，逆に不足するメディエータの補充療法を行うことになるかもしれない．

3. 将来は遺伝子操作による感染症の予防や発症時の軽症化が可能になる

更に，将来的には，遺伝子多型の部位を遺伝子導入によって操作することにより感染症に罹患しにくくしたり，発症時に重篤化しないようにすることも可能かもしれない．

おわりに

以上のように，遺伝子多型情報は臨床での非常に有用な情報である．一般市民の理解と協力のもと，日本人での信頼でき得る遺伝子多型情報を得るべく，多施設での多数の症例による共同研究が必要である．患者のプライバシー保護のもとに，個々の患者ごとに信頼し得る遺伝子多型情報に基づいた，より適切な医療が行われることが望まれる．

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根拠から学ぶ

高齢者の外科手術と術前・術後ケア

最終回　高齢者の集中治療

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はじめに

高齢社会の訪れと共に，ICU患者もますます高齢化してきた。高齢者は主な疾患のほかに種々の合併症を併存している場合が多く，また，明確な合併症がない場合でも，加齢に伴う臓器機能の低下が存在する。このように，高齢者には若年者にはない種々の特徴があるため，集中治療管理においては，高齢者に特有のポイントを理解することが大切である。

本稿では，その要点を紹介する。

1．高齢者のICU管理のポイント（表1）

高齢者の術後ICUでの管理の要点は，組織の再生を図るのに十分な組織酸素供給量を維持し，疼痛を制御することである。高齢者では，各種臓器の予備力が低下しており，循環血液量をはじめ，生体機能維持のための許容範囲が狭い。モニタリングを頻回に行い，水分や電解質などの補正は，若年者の場合よりも時間をかけて行うことが大切である。

表1　高齢者のICU管理におけるポイント

呼吸・循環管理	栄養管理
水分・電解質などの補正には時間をかける	疼痛対策
薬剤投与量	せん妄対策，日内リズム

2．呼吸管理

加齢に伴う呼吸機能の低下は，胸壁と肺に由来する。脊柱後弯症や脊椎の破壊は胸壁のコンプライアンスを低下させ，肋間筋の拘縮や肋軟骨の石灰化は肋骨の可動制限を生じる。また，呼吸筋の筋力低下は最大吸気／呼気力の低下をもたらす。一方，肺の弾性は低下し，コンプライアンスが増加する。そして，微小な呼吸細気管支や肺胞は虚脱し，不均衡な肺胞換気やエアトラッピングを生じる。これにより換気血流不均衡が生じ，酸素化能が約0.3～0.4mmHg/年低下する。また男性の場合，努力肺活量は14～30mℓ/年，1秒量は23～32mℓ/年低下する。さらに，低酸素や高二酸化炭素血症に対する反応性も低下する。

高齢者では，このような呼吸機能の低下により排痰困難となり，無気肺や肺炎などの呼吸器合併症を生じることが多い。これが契機となって多臓器機能

障害（MODS），多臓器機能不全（MOF）に陥る場合があり，呼吸管理は術後の重要課題である。慢性閉塞性肺疾患（COPD）を合併する高齢者では予後不良であるが，これは加齢よりも呼吸器疾患そのものの重篤度に比例する。

また，嚥下機能の低下や咳反射の低下により，口腔咽頭の細菌叢が乱れ，誤嚥の機会が増え，さらにT細胞機能や粘液のクリアランスの低下によって，肺炎の頻度や重篤度が増加する。

3．循環管理

加齢に伴い心筋細胞数が減少し，コラーゲンが増加するため，心室のコンプライアンスが減少する。自律神経組織が結合組織や脂肪に置換され線維化することにより，洞内やHis束の伝導障害を生じるため，高齢者では洞不全症候群，心房性不整脈，脚ブロックの頻度が高い。また，動脈硬化に伴い血管が硬化し，収縮期血圧の上昇を認め，心室肥大を招く。

また，加齢に伴い最高心拍数，最大心拍出量，最大酸素供給量が低下する。若年者は，運動時は心拍数の上昇により対応しているが，高齢者では1回心拍出量や心臓前負荷を増大させて対処しているため，高齢者では少量の血液量減少でも心機能低下を招く。

さらに，心筋虚血などの場合でも，症状が典型的でないことが多い。有名なFramingham Studyでは，心筋梗塞と認識されなかったり無症状であったりした割合が，45～54歳の患者では20％未満であることに対し，75～84歳の患者では40％を超えていたと報告されている[1]。

高齢者では，過剰あるいは急激な輸液により肺水腫を生じやすく，適正な循環血液量の範囲が狭いため，注意深いモニタリングが必要である。体重，尿量，In/Outバランス，血圧，脈拍，CVP（中心静脈圧）などを，若年者よりも頻回にモニタリングすることが大切である。

急性期に間質へ移行した水分が血管内へ戻ってくるリフィリングに至るまでに，高齢者では若年者の4倍以上時間を要するという報告もある[2]。しかし高齢者の心臓では前負荷がある程度必要であるため，循環血液量の不足は避けなければならない。循環血液量の不足は腎と冠動脈の血流量を減少させ，組織酸素供給が障害され，腎不全や心筋虚血を来す。

高齢者は頻脈にも弱い。心房細動による心房の充満不足や頻脈による左心室の充満時間の短縮は，心拍出量を低下させる。

加齢によってもα作用性機能は変わらないが，β作用性機能は低下し，筋収縮力や脈拍調節作用，β拮抗薬に対する血管拡張作用の低下を来す。また，塩酸ドブタミンに対する反応性も低下する。高齢者では，加齢に伴って拍出路が硬化しているため，後負荷の軽減はより有効である。

4．薬剤投与量

加齢に伴い，糸球体が硬化し，細動静脈の萎縮，腎尿細管細胞数の減少が起こる。腎血流は半減し，糸球体濾過量（GFR）も80歳では45％減少し，これに伴い，クレアチニンクリアランスは0.75mℓ/年低下すると言われている。薬剤はいくつかの経路で代謝されるが，多くの薬剤はGFRに比例するため，高齢者では使用薬剤の種類や量の調節が必要である。

血清クレアチニンを用いたCockcroftとGaultの公式[3]では，クレアチニンクリアランスの予測式は次のように示されている。

[(140－年齢)×体重(kg)]/[72×クレアチニン(mg/dℓ)]

重症患者や腎機能に直接影響する薬剤を使用している患者の場合には，この公式やクレアチニンクリアランスの実測値，薬剤血中濃度を測定して投与量を決定する。また，重篤時には肝・腎機能の低下を伴うことも多く，薬剤投与量はこれらによる代謝速度の低下を考慮の上で決定する。

5．栄養管理

高齢者では，低栄養や栄養失調の比率が高く，栄養管理には若年者以上に気をつけなければならない。通常の成人患者での高カロリー輸液は合併症を増加させるため，漫然と行うのではなく，腸管機能が維持されていれば経腸栄養を行う。高齢者では経腸栄養を早期に始めた方が，合併症発生率や入院期間，医療費の軽減をもたらし，QOLが向上することが示されている[4]。ただし，術前からの，あるいは手術に伴う嚥下機能や咳漱反射の低下を認める場合があるため，経腸栄養を施行する場合には誤嚥性肺炎に注意する。ベッドを30°ほどのファウラー位にすることが，誤嚥防止に有効であるとの研究がある。

高齢者における血糖コントロールは若年者より困難なことが多いが，筋肉量の減少から必要カロリーは若年者より少なく，80歳では40歳より15％低いと言われている。一方，必要タンパクは減少せず，むしろ増加すると言われるが，加齢に伴う腎機能の低下から，非タンパクカロリーに対するアミノ酸投与量の割合（カロリー/N比）を成人の健常者（150～200）より高くした方がよいことが多く，症例ごとに検討する。

6．疼痛対策

疼痛はストレスであり，不適切な対処は術後経過に重篤な影響を与える。疼痛は頻脈，心筋酸素消費量の増加を招き，心筋虚血を来す可能性がある。また，加齢に伴う呼吸機能の変調に加え，疼痛のために排痰を我慢することで，無気肺や肺炎をさらに生じやすくなる。その防止のため，排痰が可能になるように十分な疼痛対策を行うことが必要である。一方，過度の鎮静による呼吸停止，換気不全，誤嚥性肺炎を来さないように留意することも必要である。

疼痛は精神症状の変容などを来す誘因ともなる。疼痛程度の数値化やビジュアルアナログスケール（VAS）は有用であるが，もともと痴呆がある患者，せん妄がある患者，挿管中の患者では使用しづらい。また，除痛がしっかりされていないこと自体が興奮や混迷を来す。言語による意思疎通が図れない場合でも，脈拍，血圧の上昇，精神の興奮や顔貌，ボディランゲージによって疼痛の程度を確認し，鎮痛を図る。

術後はモルヒネなどの麻薬を使用する場合が多い。投与ルートは非常に重要で，高齢者では硬膜外からの麻薬による鎮痛が術中・術後の疼痛対策に有用であり，鎮静の必要性を減少させる[5]。最近は持続皮下・筋肉内注射が登場し，患者が投与量をコントロールするPCA（patient-controlled analgesia）も行われるようになった。多数の無作為化比較対照試験（RCT）で，高齢者においてもPCAにより合併症の軽減，鎮静の減少，患者満足度の向上が示されている。

7．せん妄対策，日内リズム

痴呆の頻度は65～70歳では1.5％であるが，その後5年ごとに倍増し，85歳以上では25％近くとなる。術前からの痴呆は，術後のせん妄のリスク因子である。しかし，日常は健常な場合でも，手術によるストレス，急変，入院などにより，70歳以上の患者では認識力の低下やせん妄を生じさせる。視力，聴力などの低下に伴い，環境の変化への順応力が低下

し，病院などの慣れない環境では，たとえ短期間であっても混乱し，うつとなり，さらに状態が悪化する。せん妄のリスクファクターを表2に示す。

これを防止するためには，十分な除痛，睡眠に心がける。また，ICU内でも日夜のリズムをつけるために，照明や音量を調整し，深夜の薬剤投与や経腸栄養などを避けるのが望ましい。また，患者の近くに時計やカレンダーを置き，可能であれば眼鏡や補聴器を使用し，日常生活に近い生活環境を整えることが大切である。家族にできるだけ面会してもらい，早期離床や食事介助に協力してもらうとよい。

8．終末期と集中治療

高齢のため，あるいは患者がすでに寝たきりや痴呆状態であるために，患者・家族が積極的な治療を望まず，DNAR（do not attempt resuscitation）を表示する場合もある。また，癌や疾患の重症度と加齢に伴う予備力の低下を考慮し，回復の可否の判断もしなければならない。このような場合には，患者・家族が十分納得し，満足できるように，医療従事者はコミュニケーションを十分に取ることが必要である。特に患者・家族は医師には遠慮して本心を話さない場合もあり，看護師に初めて心情や意向を打ち明けることも多く，看護師は医療従事者側の最大のキーパーソンである。

表2　せん妄のリスクファクター

入院時	補正相対危険度（95%信頼区間）
視力低下	3.51（1.15 ～ 10.71）
重症度	3.49（1.48 ～ 8.23）
MMSE＜24	2.82（1.19 ～ 6.65）
BUN／Cre比＞18	2.02（0.89 ～ 4.60）

相対危険度：要因がある群のリスクの，要因がない群のリスクに対する比率
95%信頼区間：上限と下限の間に母集団の「真の」値が存在する可能性が95%であること

Inouye SK, Viscoli CM, Horowitz RI, et al. A Predictive model for deririum in hospitalized elderly medical patients based on admission chara cteristies. Ann Intern Med 119：474-481, 1993.より引用，一部改編

おわりに

高齢社会が進み，高齢の患者はますます増加している。高齢者に特有の術後管理のポイントを理解することは，手術の成否を左右する鍵である。しかし，まだ病態が不明であったり，有効な治療法が限られていたりする部分も多く，今後のさらなる解明や新規治療法の開発が望まれる。

しかし，患者・家族が現況を正しく理解している場合には，医療従事者がいかに患者のことを真剣に考え，診療に当たっているかによって，患者・家族の満足度は大きく変化する。十分なコミュニケーションの下に適切な医療を行うように心がけたい。

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Kupffer Cell–Derived Interleukin 10 Is Responsible for Impaired Bacterial Clearance in Bile Duct–Ligated Mice

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Extrahepatic cholestasis often evokes liver injury with hepatocyte apoptosis, aberrant cytokine production, and—most importantly—postoperative septic complications. To clarify the involvement of aberrant cytokine production and hepatocyte apoptosis in impaired resistance to bacterial infection in obstructive cholestasis, C57BL/6 mice or Fas-mutated *lpr* mice were inoculated intraperitoneally with 10^7 colony-forming units of *Escherichia coli* 5 days after bile duct ligation (BDL) or sham celiotomy. Cytokine levels in sera, liver, and immune cells were assessed via enzyme-linked immunosorbent assay or real-time reverse-transcriptase polymerase chain reaction. BDL mice showed delayed clearance of *E. coli* in peritoneal cavity, liver, and spleen. Significantly higher levels of serum interleukin (IL) 10 with lower levels of IL-12p40 were observed in BDL mice following *E. coli* infection. Interferon γ production from liver lymphocytes in BDL mice was not increased after *E. coli* infection either at the transcriptional or protein level. Kupffer cells from BDL mice produced low levels of IL-12p40 and high levels of IL-10 *in vitro* in response to lipopolysaccharide derived from *E. coli*. *In vivo* administration of anti–IL-10 monoclonal antibody ameliorated the course of *E. coli* infection in BDL mice. Furthermore, BDL-*lpr* mice did not exhibit impairment in *E. coli* killing in association with little hepatic injury and a small amount of IL-10 production. In conclusion, increased IL-10 and reciprocally suppressed IL-12 production by Kupffer cells are responsible for deteriorated resistance to bacterial infection in BDL mice. Fas-mediated hepatocyte apoptosis in cholestasis may be involved in the predominant IL-10 production by Kupffer cells. (HEPATOLOGY 2004;40:414–423.)

The high incidence of perioperative infectious complications in patients with cholestasis is well documented.[1–4] Dysfunction of phagocytes and bacterial translocation from the gut due to loss of mucosal integrity are believed to be responsible for septic complication in patients with obstructive jaundice.[1,5–8] It has been reported that proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL) 6, are increased in sera without any exogenous stimuli in cholestatic conditions, suggesting that cholestasis evokes inflammatory reaction in the host.[9,10] It is also demonstrated that rats and mice with experimental obstructive jaundice produce higher levels of proinflammatory cytokines including TNF-α, IL-1, and IL-6 after lipopolysaccharide (LPS) injection compared with those without obstructive jaundice, and that cholestatic animals are susceptible to LPS-induced organ failure and mortality.[11–13] However, involvement of anti-inflammatory cytokines in host resistance to bacterial infection in cholestasis or cy-

Abbreviations: BDL, bile duct ligation/bile duct–ligated; IL, interleukin; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; IFN-γ, interferon γ; mAb, monoclonal antibody; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; HBSS, Hanks' balanced salt solution; ELISA, enzyme-linked immunosorbent assay; mRNA, messenger RNA.

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tokine profile in response to exogenously administered viable bacteria in cholestatic animals remains to be elucidated.

IL-10, an anti-inflammatory cytokine, was first described as having an ability to protect mice from LPS-induced fatal shock by suppressing proinflammatory cytokine production, including TNF-α and interferon (IFN) γ.[14] On the other hand, it has also been shown that IL-10 hampers host defense mechanisms against microbial infection by suppressing macrophage functions.[15] These contrary findings suggest that homeostasis during bacterial infection is maintained through a delicate balance between pro- and anti-inflammatory cytokines. It would thus appear that IL-10 might be involved in the immune dysfunction in cholestasis.

It has been demonstrated that macrophages are made capable of producing IL-10 after engulfing apoptotic cells in general.[16,17] Because IL-10 is shown to inhibit apoptosis pathways in a variety of cells, including hepatocytes, IL-10 production may play an important role in terminating cell death, including apoptosis, thereby suppressing excessive inflammatory reaction.[18] Bile duct ligation (BDL) evokes liver injury with hepatocyte apoptosis in mice.[19] It has been demonstrated that liver sinusoidal cells such as Kupffer cells and endothelial cells remove apoptotic hepatocytes induced by various stimuli, including lead nitrate, cycloheximide, and ultraviolet radiation.[20–23] From these findings, it is possible to speculate that Kupffer cells may become capable of producing IL-10 predominantly as a result of ingesting increased apoptotic hepatocytes in cholestatic liver.

The overall objectives of this study were to elucidate the underlying mechanisms for impaired bacterial clearance in cholestasis, focusing on pro- and anti-inflammatory cytokines and to determine if aberrant cytokine production in BDL mice after *Escherichia coli* infection is dependent on Fas-mediated apoptosis of hepatocytes. We found that Kupffer cells but not peritoneal macrophages produced a large amount of IL-10 after *E. coli* infection in mice with cholestasis and that predominant IL-10 production by Kupffer cells was associated with hepatocyte apoptosis. Our data may provide new insight into the pathogenesis of bacterial infection in cholestasis.

Materials and Methods

Mice and Microorganisms. Eight- to 10-week-old female C57BL/6 mice and *lpr/lpr* mice with nonfunctional Fas expression with C57BL/6 background were purchased from Japan SLC (Hamamatsu, Japan). All mouse experiments were approved by the University Committee on Animal Research and received humane care in accordance with National Institutes of Health publication 86-23 (*Guide for the Care and Use of Laboratory Animals*).

E. coli (ATCC No. 26) grown in Trypto-soya broth (Nissui, Tokyo, Japan) was washed repeatedly, resuspended in phosphate-buffered saline, and stored at −80°C. The concentration of bacteria was quantitated by plate counts.

Reagents. LPS from *E. coli* (serotype B6: O26) was obtained from Sigma Aldrich (St. Louis, MO). 2.4G2 (anti–FcRII/III-specific monoclonal antibody [mAb], rat immunoglobulin G_1, producing hybridoma) was obtained from American Type Culture Collection (Manassas, VA). Phycoerythrin-conjugated anti-B220 and anti-CD11b mAb, biotin-conjugated anti-Gr.1 and NK1.1 mAb, fluorescein isothiocyanate–conjugated anti-CD3 mAb, and Cy-chrome-conjugated streptavidin were purchased from PharMingen (San Diego, CA). Rat immunoglobulin G anti–mouse IL-10 mAb was purchased from R&D Systems, Inc. (Minneapolis, MN). Control isotype rat immunoglobulin G was purchased from Sigma.

Surgical Procedure. After 7 days of acclimation, surgery was performed under sterile conditions. Mice were anesthetized via intraperitoneal pentobarbital injection (50 mg/kg). An abdominal midline incision was made, and the common bile duct was ligated and divided as described previously.[2] Control animals underwent a sham procedure in which the common bile duct was exposed but not ligated.

Histological Studies. Liver specimens were removed and fixed with 10% buffered formalin, paraffin-embedded, and stained with hematoxylin-eosin for light microscopic examination. *In situ* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay was performed using an *in situ* apoptosis detection kit (Apoptag, Intergen, Purchase, NY). All steps were performed according to the instructions of the manufacturer.

Assay for Serum Bilirubin Levels and Alanine Aminotransferase Activity. Serum total bilirubin levels were measured using a commercially available kit following the manufacturer's instructions (Sigma Dianostics Kit no. 550 for bilirubin). Serum alanine aminotransferase activity was determined using the aminotransferase test kit (Wako, Osaka, Japan).

Preparation of Peritoneal Macrophages. Peritoneal exudate cells were obtained from peritoneal cavity via lavage with 3 mL of Hanks' balanced salt solution (HBSS). Peritoneal exudates were centrifuged at 110*g* for 5 minutes, washed twice, and resuspended at optimal concentrations in Dulbecco's Modified Eagle Medium (Gibco, Grand Island, NY) supplement with 10% fetal bovine

serum. Peritoneal exudate cells were spread on plastic plates and incubated for 1 hour in a CO_2 incubator at 37°C to obtain adherent cells.

Preparation of Kupffer Cells. Kupffer cells were isolated from sham and BDL mice by collagenase digestion and differential centrifugation using Percoll (Pharmacia, Uppsala, Sweden) as described elsewhere[24,25] with slight modifications. Briefly, the liver was perfused *in situ* through the portal vein with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline containing 10 mM ethylenediaminetetraacetic acid at 37°C for 5 minutes. Subsequently perfusion was performed with HBSS containing 0.1% collagenase IV (Sigma) at 37°C for 5 minutes. After digestion, the liver was excised and the suspension was filtered. The filtrate was centrifuged twice at 50*g* at 4°C for 1 minute. The supernatant was collected and centrifuged at 300*g* for 5 minutes, and the pellet was resuspended with buffer. The cell suspension was then layered on top of a density cushion of 25%/50% discontinuous Percoll (Pharmacia) and centrifuged at 900*g* for 20 minutes to obtain the Kupffer cell fraction, followed by washing with the buffer again. Cells were plated in 6-cm plastic culture dishes (FALCON, Becton Dickinson, NJ) and cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum and 10 mM hydroxyethylpiperazine-N-2 ethanesulfonic acid. After incubation for 30 minutes, nonadherent cells were removed, cold Ca^{24}- and Mg^{2+}-phosphate-buffered saline with 10 mM ethylenediaminetetraacetic acid was added, and the cells were put on ice for 40 minutes. After tapping the dish gently, the supernatant was collected and centrifuged at 300*g* for 5 minutes. The pellet was resuspended with 1×10^6 cells/mL in RPMI and immediately used. The purity and cell viability of Kupffer cells isoplated were more than 91% and 95% as assessed by phagocytotosis of latex beads and trypan blue exclusion, respectively (data not shown).

Preparation of Liver Lymphocytes. Fresh liver was immediately perfused with sterile HBSS through the portal vein and then meshed with stainless steel mesh. After the coarse pieces were removed by centrifugation at 50*g* for 1 minute, the cell suspensions were again centrifuged, resuspended in 8 mL of 45% Percoll (Pharmacia), and layered on 5 mL of 66.6% Percoll. The gradients were centrifuged at 600*g* at 20°C for 20 minutes. Lymphocytes at the interface were harvested and washed twice with HBSS.

Bacterial Growth in Organs. After infection, peritoneal exudates were obtained from the peritoneal cavity by lavage with 3 mL of HBSS. Serial dilutions of the exudate samples were plated to determine the number of viable bacteria. For the enumeration of viable bacteria in the liver, the liver was perfused with 8 mL of sterile HBSS to wash out bacteria in the blood vessels immediately after mice were bled. The liver and spleen were removed and separated into sterile Teflon-coated homogenizers (Asahi Techno Glass Co., Tokyo, Japan) containing 2 mL of cold phosphate-buffered saline. After each organ was homogenized thoroughly, the bacterial counts in the homogenates were established by plating serial 10-fold dilutions in sterile distilled water on tryptic soy agar (Nissui). Colonies were counted 24 hours after incubation at 37°C.

Serum and Peritoneal Lavage Fluid Cytokine Assays. TNF-α, IL-12, IL-10, and IFN-γ levels in serum, peritoneal lavage fluid, and culture supernatants were determined via enzyme-linked immunosorbent assay (ELISA). ELISAs were performed using Genzyme mAb according to the manufacturer's instructions (Genzyme, Cambrigde, MA).

Flow Cytometry Analysis. Peritoneal exudate cells were preincubated with a culture supernatant from 2.4 G2 to prevent nonspecific staining. For the identification of macrophages and polymorphonuclear cells, the cells were then stained with phycoerythrin-conjugated anti-CD11b mAb and biotinylated anti-Gr.1 mAb. For the identification of lymphocytes, the cells were stained with fluorescein isothiocyanate–conjugated anti-CD3 mAb, phycoerythrin-conjugated B220 mAb, and biotinylated anti-NK1.1 mAb. To detect biotin-conjugated mAb, cells were stained with Cy-Chrome–conjugated streptavidin. All incubation steps were performed at 4°C for 30 minutes. The stained cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The data were analyzed using FACSCalibur research software (Becton Dickinson).

Expression of IL-10, IL-12, TNF-α, and IFN-γ Genes in Liver Homogenates, Liver Lymphocytes, Kupffer Cells, and Peritoneal Macrophages. Total RNA was extracted from liver homogenates, liver lymphocytes, Kupffer cells, and peritoneal macrophages using TRIzol reagent (Life Technologies, Rockville, MD). Complementary DNA was synthesized from 2 μg of total RNA by reverse transcription.[26] Real-time polymerase chain reaction was performed with the SYBR Green PCR Master Mix and ABI PRISM 7700 Sequence Detection Systems (Applied Biosystems, Foster City, CA) according to the manufacturer's suggested protocol. The specific primers were as follows: IL-12p40 sense, 5′-CGTGCTCATGGCTGGTGCAAAG- 3′; IL-12p40 antisense, 5′-CTTCATCTGCAAGTTCTTGGGC- 3′; IL-10 sense, 5′-CCAGTTTTACCTGGTAGAAGTGATG- 3′; IL-10 antisense, 5′-AACTCAGACGACCTGAGGTCCTGGATCTGT-3′; IFN-γ sense, 5′-AGCGGCTGACTGAACTCAGATTGTAG-3′; IFN-γ antisense, 5′-

GTCACAGTTTTCAGCTGTATAGGG-3′; TNF-α sense, 5′-GGCAGGTCTACTTTGGAGTCATTGCCCC-3′; TNF-α antisense, 5′-ACATTCGAGGCTCCAGTGAATTCGG-3′; β-actin sense, 5′-TGGAATCCTGTGGCATCCATGAAAC-3′; and β-actin antisense, 5′-TAAAACGCAGCTCAGAACAGTCCG-3′.

In Vitro *Cytokine Production of Peritoneal Macrophages and Kupffer Cells.* Purified peritoneal macrophages in Dulbecco's Modified Eagle Medium (2×10^6/mL) or Kupffer cells in RPMI 1640 (1×10^6/mL) were harvested in 96-well culture plates and stimulated with LPS (0.1 μg/mL) for indicated times. Supernatants were harvested at 0, 3, 6, 10, and 16 hours. TNF-α, IL-12p40, and IL-10 concentrations of supernatants were measured using ELISA.

In vitro *IFN-γ Production of Cultured Liver Lymphocytes.* Freshly isolated liver lymphocytes were harvested 6 hours after *E. coli* infection. Liver lymphocytes in RPMI (5×10^6/mL) were cultured *in vitro* for 24 hours without additional stimulation. Culture supernatants were harvested and analyzed for IFN-γ content using ELISA.

Statistical Analysis. All data are presented as means ± SD. Data were analyzed for significance using Student's *t* test, and a Bonferroni correction was applied for multiple comparison. A value of $P < .05$ was considered statistically significant.

Results

Fas-Dependent Hepatocyte Apoptosis in Obstructive Jaundice. Five days after BDL, serum bilirubin levels reached 13.2 ± 4.6 mg/dL and remained at the plateau thereafter in C57BL/6 mice. Histological examination revealed that liver in BDL mice exhibited infrequent focal necrosis and cellular infiltration with marked bile duct proliferation, whereas liver of sham-operated mice showed almost normal appearance (Fig. 1A and 1B). For all subsequent experiments, mice undergoing BDL for 5 days were used. Apoptotic cells were next identified using the TUNEL technique. After 5 days of BDL, hepatocytes undergoing apoptosis could be observed (Fig. 1B and 1E). In contrast, the number of TUNEL-positive cells remained low in control mice and BDL-*lpr* mice (Fig. 1D and 1F). These results suggest that BDL induces hepatocyte injury, including Fas-dependent apoptosis.

Increased Susceptibility of BDL Mice to* E. coli *Infection. To evaluate bactericidal activity of BDL mice, we examined the kinetics of bacterial growth in peritoneal cavity, liver, and spleen after intraperitoneal inoculation with *E. coli* (1×10^7 colony-forming units/mouse). As shown in Fig. 2, the bacterial counts were significantly higher at any time point after *E. coli* infection in BDL mice than in sham-operated mice ($P < .05$). Because these results were consistent with previous reports,[5,27,28] we concluded that bacterial killing is severely impaired in BDL mice.

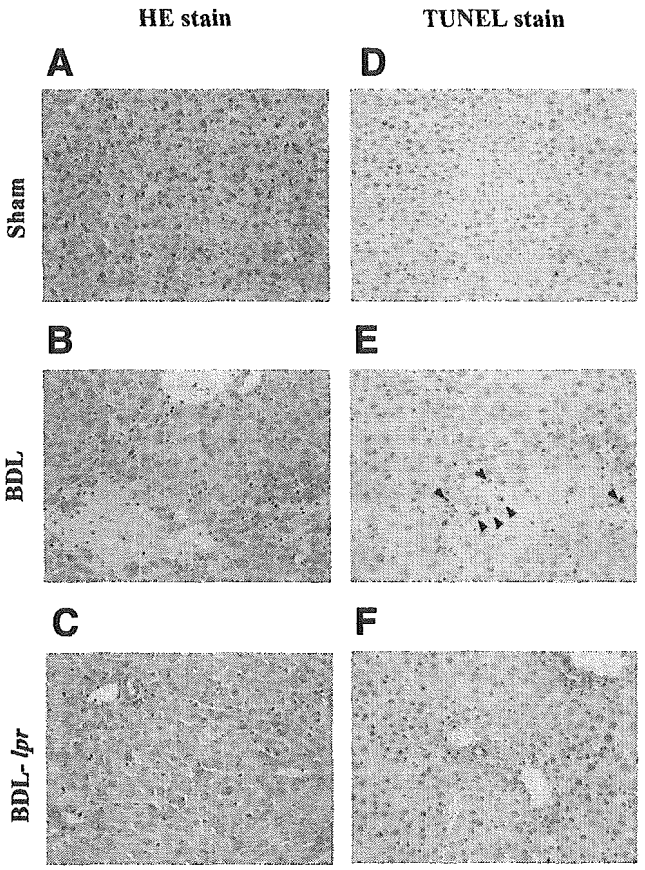

Fig. 1. Representative histological sections of the liver on the 5th postoperative day. (A-C) Hemotoxylin-eosin stain. (D-F) TUNEL stain. (A, D) Sham-operated mouse. (B, E) BDL mouse. (C, F) BDL-*lpr* mouse. Bile duct proliferation and cellular infiltration were seen in both wild type and *lpr* mice after BDL, whereas focal spotty necroses were observed only in wild type mice with BDL. Increased numbers of TUNEL-positive cells (**arrowheads**) were observed in the liver of BDL mice in contrast to sham-operated or BDL-*lpr* mice. Data shown are representative of 3 independent experiments. HE, hemotoxylin-eosin; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling; BDL, bile duct ligation.

Emergence of Peritoneal Exudate Cells After* E. coli *Infection in BDL Mice. A prominent increase in polymorphonuclear cells, which are thought to be responsible for rapid elimination of the bacteria, was observed in the peritoneal cavity after an intraperitoneal infection with *E. coli*. To elucidate the cause for deteriorated exclusion of bacteria in BDL mice, we examined the influx of phagocytes in the peritoneal cavity after *E. coli* inoculation. There was no substantial difference in numbers of polymorphonuclear cells, lymphocytes, or macrophages in the peritoneal cavity between BDL and sham-operated mice

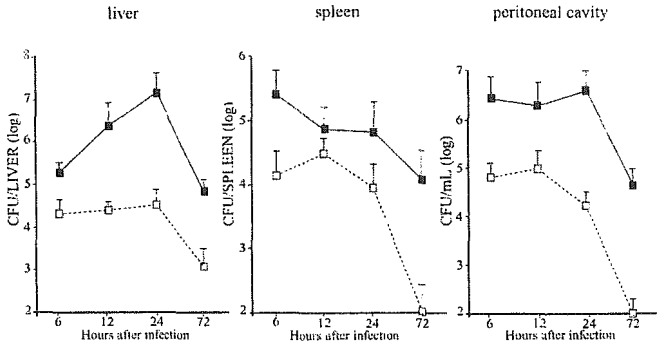

Fig. 2. Delayed clearance of *E. coli* in liver, spleen, and peritoneal cavity in BDL mice. Data are representative of 4 separate experiments and are expressed as the mean ± SD for 5 mice in an experiment. (■) BDL mice. (□) Sham-operated mice. CFU, colony-forming units.

at 6 hours after *E. coli* infection when a large difference in number of viable bacteria was seen (Fig. 3). The numbers of polymorphonuclear cells and macrophages were significantly larger at 24 hours in BDL mice after *E. coli* infection, presumably due to increased bacterial burden at this stage. These results indicate that accumulation of phagocytes is not impaired in BDL mice after *E. coli* infection.

Aberrant Cytokine Production in BDL Mice After* E. coli *Challenge. Cytokine production was examined in the sera of sham-operated and BDL mice after *E. coli* infection. As shown in Fig. 4A, serum TNF-α and IL-10 levels were maximal at 1 hour after *E. coli* infection, while the IL-12 level reached a peak at 3 hours after infection in both BDL and sham-operated mice. Serum IL-10 levels were significantly higher in BDL mice than in sham-operated mice, while increases in IL-12 levels were significantly suppressed in BDL mice ($P < .05$). There was no significant difference in serum TNF-α level between sham-operated and BDL mice. The patterns of cytokine profile of peritoneal lavage fluid were similar to those of the serum (Fig. 4B). Neither IL-4 nor IFN-γ was detected in the sera or peritoneal lavage fluids of either BDL or sham-operated mice at any stage after *E. coli* infection (data not shown). These results clearly indicated that mice with obstructive jaundice present predominant IL-10 production over IL-12 after *E. coli* infection.

IFN-γ Production By Liver Lymphocytes of BDL Mice After* E. coli *Infection. To further investigate the cytokine profiles in BDL mice, we next examined messenger RNA (mRNA) expression in the whole liver homogenates of BDL mice or sham-operated mice after *E. coli* infection. Consistent with serum cytokine levels, high expression of IL-10 mRNA together with low expression of IL-12p40 mRNA was seen in BDL mice. Notably, expression of IFN-γ mRNA was not increased in liver of BDL mice after *E. coli* infection (Fig. 5A).

Because IFN-γ is necessary for macrophages to be activated so that they may kill microorganisms, we next determine the quantitative difference in IFN-γ production by liver lymphocytes between BDL and sham-operated mice. The expression of IFN-γ mRNA remained suppressed in liver lymphocytes isolated from BDL mice at any time point after *E. coli* infection compared with those from sham-operated mice (Fig. 5B). Moreover, whereas liver lymphocytes isolated from sham-operated mice 6 hours after *E. coli* infection produced a high level of IFN-γ *in vitro* without additional stimulation, IFN-γ production was barely detectable in lymphocytes from BDL mice (Fig. 5C).

Differences in Cytokine Production Between Kupffer Cells and Peritoneal Macrophages of BDL Mice. To seek the source of serum cytokines produced after *E. coli* inoculation, we compared cytokine production by Kupffer cells with that by peritoneal macrophages in response to LPS derived from *E. coli* in vitro. The peritoneal macrophages and Kupffer cells isolated from BDL or sham-operated mice were stimulated *in vitro* with LPS, and the concentrations of TNF-α, IL-12, and IL-10 in the culture supernatants were determined via ELISA. As shown in Fig. 6A, the peritoneal macrophages of BDL mice produced considerably higher levels of TNF-α but significantly lower levels of IL-10 3 hours after LPS stimulation than those of sham-operated mice. The level of IL-12p40 production was higher in the peritoneal macrophages of BDL mice at 10 hours and 16 hours after LPS stimulation compared with those of sham-operated mice.

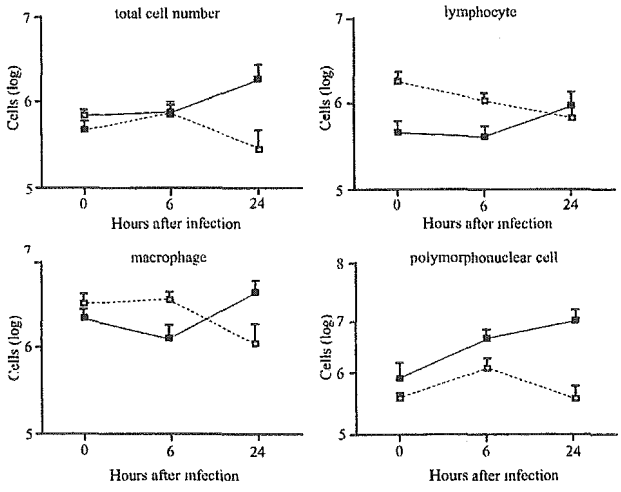

Fig. 3. Kinetics of peritoneal exudate cells of sham-operated (□) and BDL (■) mice after intraperitoneal inoculation with 10^7 colony-forming units of *E. coli* were analyzed with flow cytometry. Accumulation of immune cells after *E. coli* infection was not impaired in BDL mice. Data are representative of 3 separate experiments and are expressed as the mean ± SD for 4 mice in an experiment.

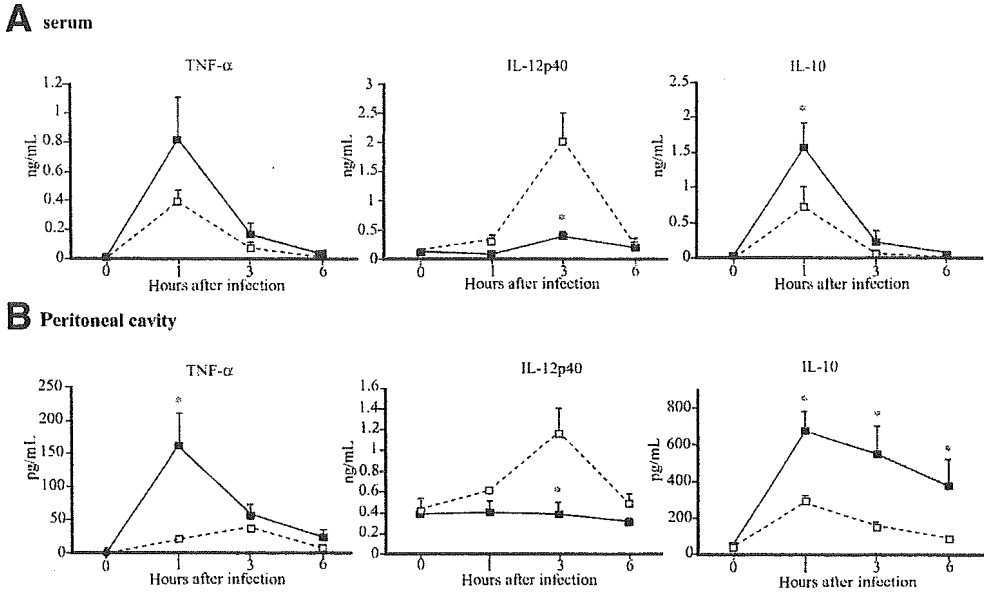

Fig. 4. Kinetics of cytokine levels in (A) serum and (B) peritoneal lavage fluid after *E. coli* challenge. Plasma and peritoneal lavage fluid samples were obtained at the indicated time points after intraperitoneal inoculation with 10^7 colony-forming units of *E. coli*. TNF-α, IL-12, and IL-10 concentrations were determined via ELISA. Data are representative of 3 separate experiments and are expressed as the mean ± SD for 5 mice in an experiment. *$P < .05$ versus the sham-operated group. (■) BDL mice. (□) Sham-operated mice. TNF-α, tumor necrosis factor α; IL, interleukin.

On the other hand, Kupffer cells from BDL mice, in response to LPS, produced a larger amount of IL-10 and a smaller amount of IL-12 compared with those from sham-operated mice (Fig. 6B). There was no difference in TNF-α production by Kupffer cells between BDL and sham-operated mice.

We further compared mRNA expression for IL-10, IL-12p40, and TNF-α in Kupffer cells and peritoneal macrophages between BDL and sham-operated mice after LPS stimulation. As shown in Fig. 5D and 5E, IL-10 mRNA was increased in Kupffer cells of BDL mice after LPS stimulation, whereas the peritoneal macrophages of BDL mice expressed less IL-10 mRNA than those of sham-operated mice. Increase in IL-12p40 mRNA was only marginal in Kupffer cells of BDL mice but prominent in the peritoneal macrophages of BDL mice after LPS stimulation. TNF-α mRNA increased more markedly in both Kupffer cells and peritoneal macrophages of BDL mice after LPS stimulation compared with those in sham-operated mice. These results indicate that peritoneal macrophages and Kupffer cells from BDL mice respond differently to LPS in terms of IL-10 and IL-12 production and that the cytokine profiles of peritoneal macrophages are similar to those of Kupffer cells in sham-operated mice.

Effect of IL-10 Neutralization on Resolution of* E. coli *Infection. Because IL-10 is known to hamper the resolution of bacterial infection in mice, we next determined whether or not increased IL-10 production in BDL mice is responsible for the impaired host defense against *E. coli* infection. BDL mice were injected intraperitoneally with anti–IL-10 neutralizing mAb (200 μg/mouse) 2 hours before *E. coli* challenge, and the number of the bacteria in organs was examined 24 hours after infection. As shown in Fig. 7, impaired bactericidal activity was reversed by administration of anti–IL-10 mAb in BDL mice. These results suggest that early IL-10 production by Kupffer cells might be responsible for hampered resolution of *E. coli* infection in BDL mice.

IL-10 Production in Fas-Mutated BDL Mice After* E. coli *Infection. As demonstrated above, we found that BDL induced predominant IL-10 production specifically by Kupffer cells in mice. We hypothesized that Kupffer cells in BDL mice have been changed to readily produce IL-10 as a result of ingesting apoptotic hepatocytes, because it is demonstrated that macrophages produce IL-10 after ingesting apoptotic cells to prevent unnecessary immune responses.[16,17] To investigate this possibility, Fas-mutated *lpr/lpr* (*lpr*) mice were used in the next experiment, because *lpr* mice are shown to be resistant to cholestatic liver injury, which partially involves Fas-dependent hepatocyte apoptosis. As shown in Fig. 1E, hepatocyte apoptosis was seen in liver of BDL mice, whereas few apoptotic hepatocytes were observed in liver of *lpr* mice undergoing BDL (Fig. 1F); this is consistent with previous reports.[19,29] There was no difference in serum total bilirubin levels between wild type and *lpr* mice after BDL, but serum alanine aminotransferase activity of *lpr* mice was significantly lower in *lpr* mice than in wild type mice (42.2 ± 12.4 IU/L vs. 85.1 ± 15.6 IU/L; $P < .05$). As expected, serum IL-10 level of BDL-*lpr* mice after *E. coli* infection was comparable to that of sham-operated mice (Fig. 8B). Surprisingly, the bacterial number in organs 24 hours after *E. coli* infection was significantly lower in BDL-*lpr* mice when compared with BDL wild type mice, whereas no significant difference in bacterial clear-

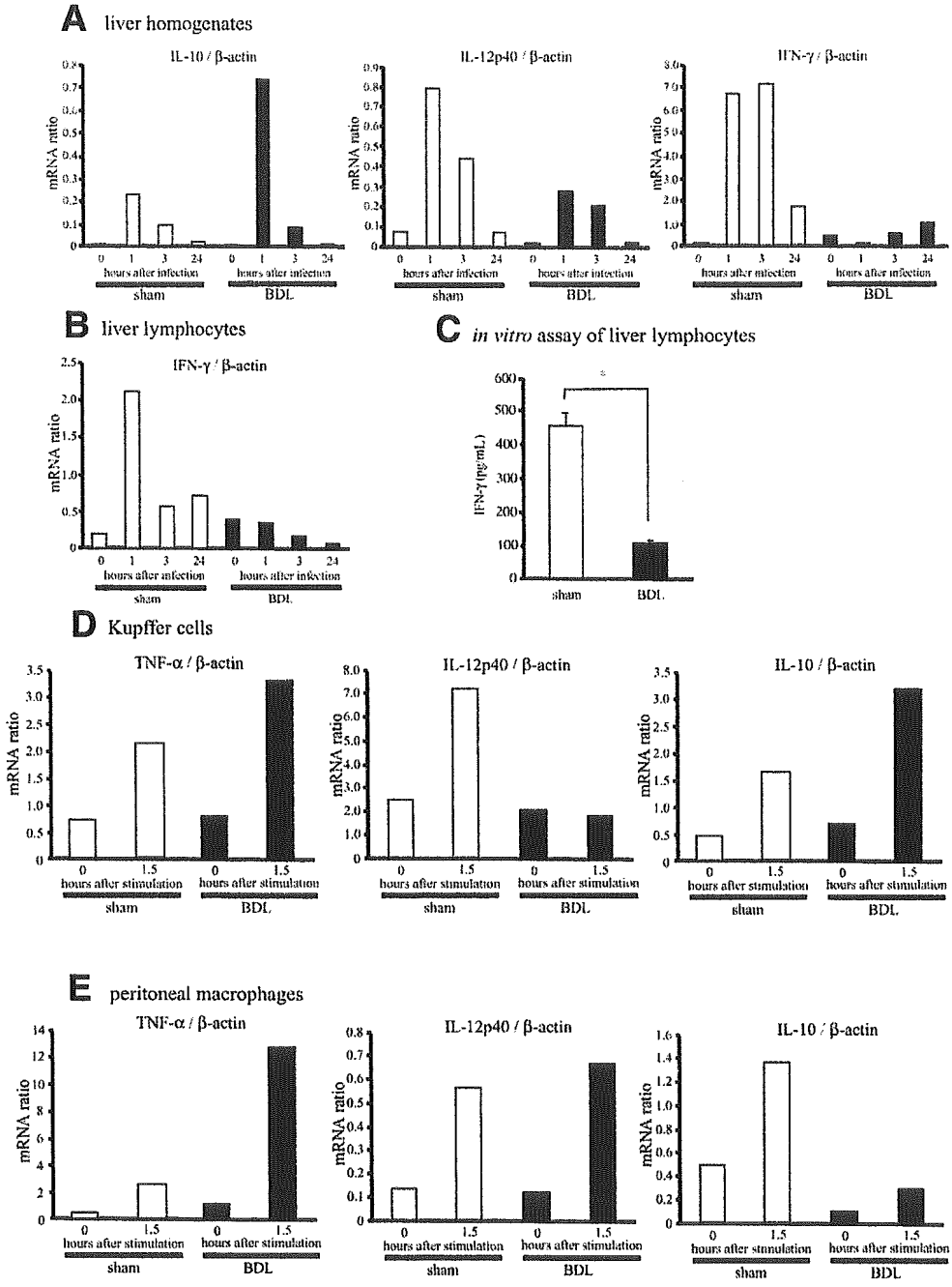

Fig. 5. Cytokine gene expressions in (A) liver homogenates and (B) liver lymphocytes after *E. coli* infection and in (D) Kupffer cells and (E) peritoneal macrophages after LPS stimulation. Cytokine gene expressions were quantitated using real-time polymerase chain reaction. Cytokine mRNA expressions of liver homogenates and liver lymphocytes were pooled from 4 mice in each group after *E. coli* infection. Cytokine gene expressions of cultured Kupffer cells and peritoneal macrophages were pooled from 5 mice in each group before and after stimulation with LPS (0.1 μg/mL). The expression of mRNA levels for each cytokines were normalized as a ratio using β-actin mRNA as a housekeeping gene. (C) *In vitro* IFN-γ production of liver lymphocytes after *E. coli* infection in sham-operated and BDL mice. Liver lymphocytes were isolated from each group before and after *E. coli* infection and cultured *in vitro* for 24 hours without additional stimulation. Culture supernatants were analyzed for IFN-γ content via ELISA. IFN-γ was not detected in culture supernatants of sham-operated or BDL mice before infection. Results were derived from 6-8 mice per group. $*P < .05$. IL, interleukin; IFN-γ, interferon γ; mRNA, messenger RNA; BDL, bile duct ligation; TNF-α, tumor necrosis factor α;

ance was seen between sham and sham-*lpr* mice (Fig. 8A). These results indicate that Fas may be partially involved in increased IL-10 production by Kupffer cells and subsequent impairment in bacterial killing in BDL mice.

Discussion

Although bile duct ligation has been shown to induce impairment in bacterial clearance, only a few reports have addressed the bactericidal activity in cholestatic animals from a standpoint of pro- and anti-inflammatory cytokines.[27,28] We show here that increased IL-10 production with decreased IL-12 release in the serum following *E. coli* infection is characteristic of BDL mice as opposed to sham-operated mice. The early IL-10 production was potentially involved in impaired resolution of *E. coli* infection because *in vivo* administration of anti–IL-10 mAb significantly augmented bacterial clearance in BDL mice. We concluded that Kupffer cells were major sources of serum cytokines because these serum cytokine levels were well correlated with those produced by Kupffer cells but not peritoneal macrophages.

T cells and natural killer cells, in the presence of IL-12, initially produce IFN-γ after bacterial infection; later increase in IL-10 suppresses IFN-γ production by these

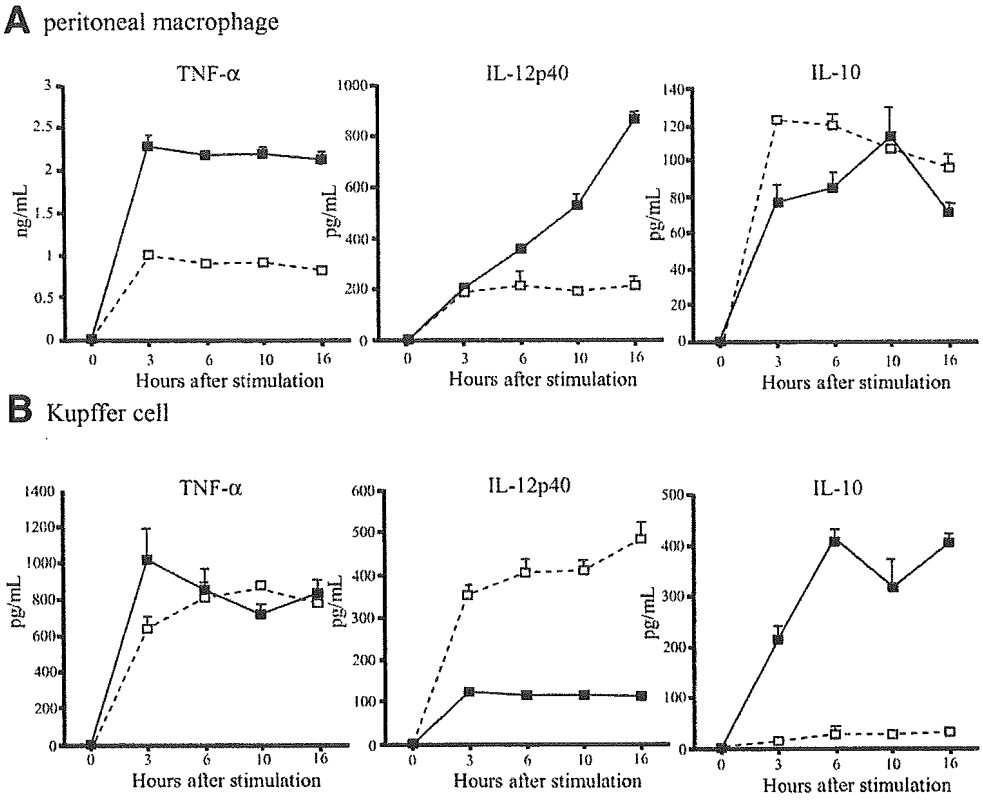

Fig. 6. Difference in cytokine profile between (A) peritoneal macrophages and (B) Kupffer cells in response to LPS *in vitro*. Kinetics of TNF-α, IL-12p40, and IL-10 production by peritoneal macrophages (2×10^6/mL) and Kupffer cells (1×10^6/mL) after stimulation with LPS (0.1 μg/mL) are shown. Culture supernatants were collected at the indicated time points. TNF-α, IL-12p40, and IL-10 concentrations were determined via ELISA. Each value represents the mean ± SD of 4 experiments. $^*P < .01$ versus the sham-operated group. (■) BDL mice. (□) Sham-operated mice. TNF-α, tumor necrosis factor α; IL, interleukin.

lymphocytes, leading to subsidance of inflammatory reaction.[14,30] We have reported previously that IFN-γ is important for bacterial clearance after *E. coli* infection in mice.[31] In fact, we have shown in the present study that liver lymphocytes of sham-operated mice expressed abundant levels of IFN-γ mRNA after *E. coli* infection, although the serum level of IFN-γ was not detected even in sham-operated mice. On the other hand, those from BDL mice expressed only a marginal level of IFN-γ mRNA. Furthermore, *in vitro* IFN-γ production was significantly higher in liver lymphocytes of sham-operated mice when compared with those of BDL mice. These data strongly suggest that the predominant IL-10 production and concomitant suppression of IL-12 production by Kupffer cells in BDL mice might be responsible for host defense dysfunction against bacterial infection.

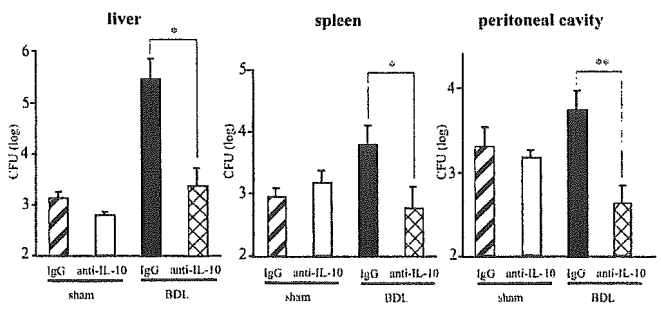

Fig. 7. The effect of neutralization of IL-10 in the course of *E. coli* infection. Sham-operated and BDL mice were injected intraperitoneally with anti-IL-10 neutralizing mAb (200 μg/mouse) 2 hours before *E. coli* challenge, and the numbers of the bacteria in liver, spleen, and peritoneal cavity were examined 24 hours after infection. $^*P < .05$; $^{**}P < .01$. CFU, colony-forming units; IgG, immunoglobulin G; IL, interleukin; BDL, bile duct ligation.

We have demonstrated that there is no significant difference in serum TNF-α production between sham and BDL mice after *E. coli* infection. In contrast, previous papers reported that BDL mice produced a large amount of TNF-α after LPS stimulation.[11,32] This may reflect a difference of LPS versus whole bacterial challenge. In fact, we found in our experimental model that serum TNF-α levels of BDL mice were more than 10 times higher than those of sham-operated mice 1 hour after 4 mg/kg LPS injection (data not shown).

A notable finding in this study is a difference in *ex vivo* production of IL-10 and IL-12 in response to LPS between Kupffer cells and peritoneal macrophages from BDL mice. We found that peritoneal macrophages from BDL mice were able to produce a large amount of IL-12 and TNF-α, whereas IL-10 production was prominent in Kupffer cells from BDL mice. These results suggest that cytokine production by Kupffer cells and peritoneal macrophages are differentially regulated by their milieu. Although serum factors in cholestasis (*e.g.*, increased concentrations of bile acids and bilirubin) are shown to affect functions of immune cells, it seems unlikely that

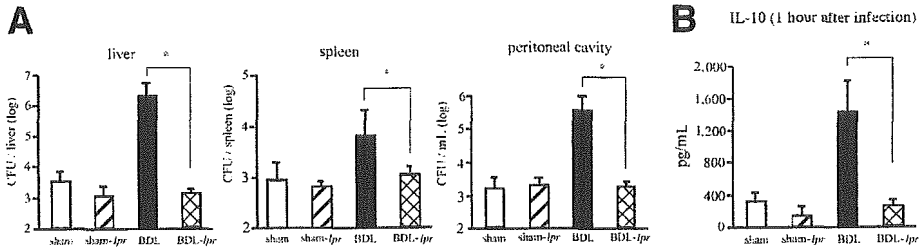

Fig. 8. Bacterial clearance in Fas-mutated *lpr* mice with BDL. (A) Bacterial clearances in organs 24 hours after *E. coli* challenge were counted in C57BL/6 and *lpr* mice. BDL-*lpr* mice did not show impaired bacterial clearance at the indicated time. Data are expressed as the mean ± SD for 5 mice. (B) Serum IL-10 production in *lpr* mice 1 hour after *E. coli* infection. Serum IL-10 level was lower in BDL-*lpr* mice compared with that in BDL mice. Results were derived from 5 mice per group. $*P < .05$. CFU, colony-forming units; BDL, bile duct ligation.

such serum factors are involved in the differential regulation of Kupffer cells and peritoneal macrophages.[1,33–35]

Kuppfer cells are shown to be activated by engulfing apoptotic hepatocytes induced by various stimuli.[20–23] Because macrophages are demonstrated to become capable of producing IL-10 after engulfing apoptotic bodies,[16,17] it is possible to speculate that Kupffer cells in cholestatic mice are also activated to generate IL-10 predominantly after ingesting increased apoptotic hepatocytes induced by BDL. To evaluate this possibility, we conducted a series of experiments using Fas-mutated *lpr* mice, which are demonstrated to be resistant to Fas-mediated hepatocyte apoptosis. We have shown that *lpr* mice are resistant to BDL-induced hepatocyte apoptosis and that *lpr* mice with BDL are able to kill *E. coli* efficiently to a similar extent to that of sham-operated mice, with a small amount of IL-10 production. These results strongly support the scenario that BDL induces predominant IL-10 production by Kupffer cells through ingesting Fas-mediated apoptosis of hepatocytes. It has also been demonstrated that Fas signaling in macrophages induces IL-10 gene expression.[36] We have recently reported that natural killer T cells in the liver express Fas-ligand directly through toll-like receptors induced by gram-negative bacteria such as *Salmonella choleraesuis* and *E. coli*.[37,38] Taken together, it also seems likely that Fas-expressing Kupffer cells in BDL mice are susceptible to signals via Fas-ligand expressed on natural killer T cells after *E. coli* infection and predominantly produce IL-10. Further studies are needed to clarify the mechanism of predominant IL-10 production by Kupffer cells in BDL mice.

In conclusion, increased IL-10 and reciprocally suppressed IL-12 production by Kupffer cells is responsible for deteriorated resistance to bacterial infection in BDL mice. Fas-mediated hepatocyte apoptosis may be involved in the predominant IL-10 production by Kupffer cells. These data support the clinical practice of biliary drainage before surgery to decrease perioperative septic complications. Moreover, our findings may provide a therapeutic approach to the control of cholestasis-associated bacterial infection.

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