犬のエキノコックス症

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Canine Echinococcosis

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感染リスクの高まり

多包条虫 *Echinococcus multilocularis* は北半球に広く分布する人獣共通寄生虫で，最近の北海道のキツネの感染率は約40%で，全道的に感染リスクが高まっている。自然界ではキツネと野ネズミの野生動物間の感染環が主であるが、人と共に生活している犬もエキノコックスの終宿主となり、飼い犬が感染すると、飼い主や住民の感染源として危険である。すでに、北海道では飼い犬・野犬から多数のエキノコックス感染例が知られており、本州へ運ばれた犬においてもエキノコックス感染例が発見された。人、家畜及び物産の交流とともにエキノコックス流行地が拡大する危険性がある。

感染状況と飼育環境

濃厚汚染地で患者数が特に多いアラスカのセントローレンス島や中国の一部の地域では、犬が人への感染源として重要である。最近の北海道では感染キツネが都市周辺でも高率に見られるようになり、野ネズミが感染し、その感染ネズミを飼い犬が食べる機会がある。

北海道における1966年から2002年までの終宿主動物の調査結果をまとめると、キツネ18.7%、イヌ1.0%、検査頭数の少ないネコとタヌキはそれぞれ5.5%、1.2%の感染率である(1)。世界中でもキツネの感染率は高いが、通常犬の感染率は低い。

世界における多包条虫の犬における感染状況は、上の表に示したように10%以上の地域もあるが、1%以下の地域もある。10%以上の地域は特に濃厚な汚染地域で、犬の放し飼いが普通で、犬と野ネズミの接点の多い地域もしくは、野犬のデータである。人の生活圏にも中間宿主となる野ネズミが生息し、犬が放し飼いにされたときなどに捕食し、多包条虫に感染するものと考えられる。北海道の飼い犬のエキノコックス検査依頼主へのアンケート調査においては、約1／4の飼い犬がネズミに興味を示すと回答している。

表　世界各地における犬の多包条虫感染状況

国	地域	年代	感染率
アラスカ	セントローレンス島	1951	12%
ロシア	ヤクーツク	1966	18% (55/307)
中国	ガンスー	1992	10.3% (6/58)
中国	スーチュワン	1991	14.3% (4/28)
中国	スーチュワン	1999	12.1 -25.0%
ドイツ	バーデン-ヴュルテンブルグ	1998	0% (0/145)
スイス	南東スイス	1999	0.30% (2/660)
スイス	フライブルグ（野犬）	1996-97	12% (5/41)
スイス	東部	1995	0.22% (2/452)
米国	ミネソタ（農家の犬）	1997	2.4% (3/123)

1983年以降の北海道（自治体）における野犬収

容所の犬の剖検調査では19頭の犬から多包条虫が検出され、そのうち14頭は野犬、４頭は飼い犬、１頭は由来不明で、これらの４頭の飼い犬はすべて放し飼いにされていた個体である。我々へ検査依頼された飼い犬の中では、10頭の多包条虫虫卵陽性犬が発見され、ラブラドール・レトリバーが3頭で、少し他の品種より高い傾向が認められた。また、陽性犬のほとんどは室外飼育で、しばしば放し飼いされている犬であった。糞便性状は正常で、軟便は一例のみに見られ、感染していても症状を示さないことが確認された。これら以外に、２例下痢で動物病院へ来院した犬において、便中に成虫が発見された例もあった。最近一年間(2003.4.1～2004.3.1）の道内の犬・猫の我々の糞便検査結果では、検査頭数1,140中、抗原陽性頭数６、抗原・虫卵ともに陽性の頭数４、虫卵DNA陽性頭数３であった。野ネズミの生息する都市周辺部や農村部で放し飼いされる犬が感染の機会が多いが、都市市街地で室内飼育でまれに郊外で放している犬でも感染が認められた。我々の今までの調査で経歴の判明している感染犬としては、拾った犬（その後室内飼)、大きな敷地や牧場内で放し飼いの犬、主に室内飼育で毎日の散歩やまれに郊外に連れて行く犬、緊急避難時に放されしばらく野犬状態の犬であった。

感染後の経過

子犬への感染実験では、投与された原頭節の内44%-87%が定着し、犬は高感受性であることが示唆された。感染後の犬における感染後の経過は糞便内寄生虫抗原の推移により推測できる。便内抗原のOD値は感染後１週間ほどで顕著に上昇し、20-40日後に減少しはじめ、2-3ヶ月までにほとんど陰転する。これらのことから寄生期間はあまり長くなく、2-3ヶ月でほとんどの虫体が排除されるものと考えられる。虫卵排泄の推移は、感染後26日から虫卵が陽転し、虫卵排泄が開始し、その後排泄虫卵数の日間変動は顕著であるが、虫卵数は減少し、2-3ヶ月でほとんど検出されなくなる(2)。寄生期間は3ヶ月前後と予想されるが、小数の虫体が長期間残存する例もある。６万個の原頭節を実験的に投与した犬では、６ヶ月間に１千万個の虫卵を排泄したと算出された。感染初期の虫卵数の日間変動は、寄生虫の発育がほぼシンクロしており、ほぼ同時に受胎片節の脱落と再生が起こるため、糞便内虫卵数の変動が起こると予想される。

終宿主の犬やキツネにおいて、成虫はその頭節で小腸粘膜に吸着するのみで、固有層に侵入したりせず、病原性は弱く、ほとんど臨床症状を示さない。犬の感染実験において、無症状で、普通の硬い便に加えて粘液の塊を排泄する程度なので、下痢便を排泄することはまれと考えられる。下痢の症例で、成虫が糞便とともに排泄された症例もあるが、小形の虫体なので、顕微鏡で観察しないと鑑別は不可能である。病理組織学的な観察では正常か、軽度のカタル性の炎症がある程度である。野外で採取されたキツネの糞便でも、正常便から虫卵が多数検出されることから、キツネは感染しても下痢などの症状は通常示さないものと推察される。中間宿主のエゾヤチネズミが非常に多数の原頭節を保有することもあるので、飼い犬が一度に百万個以上の原頭節を摂取する可能性があり、このような重度の感染時にはカタール性の腸炎を引き起こすことも考えられる。

犬に感染した場合、多包条虫に対する血清中のIgG、IgM、IgAなどの顕著な上昇があるが、感染防御における抗体の意義はまだ不明で、再感染防御も顕著ではない。

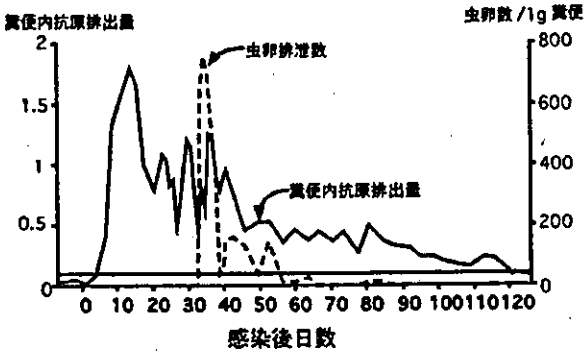
診断

エキノコックスの終宿主のための診断法がいくつか行われてきた。剖検による検査、虫卵検査、血清診断、アレコリンを用いた試験的駆虫などであるが、デメリットが多く、新たな診断法が必要とされていた。

我々はモノクロナール抗体EmA9を用い、糞便内のエキノコックス抗原をサンドイッチELISAで検出する方法を開発してきた。この方法は、糞便を加熱し殺卵後検査できることから安全で、感染後10日までに陽性となり早期診断可能で、虫卵が検出できない時期の診断も可能な方法で高感度であり、現在の感染の証明する診断法である(3)。

キツネの剖検検査（139頭、80頭虫体陽性）と

エキノコックス感染後における糞便内の抗原と虫卵数の推移



糞便の抗原検出法との相関から、感度92.5%、特異度96.6%であり、一方、虫卵検査は感度46.3%、特異度100%（この調査ではテニア科条虫としては多包条虫のみ検出された。）であった。さらに未感染犬として本州（千葉・神奈川）の飼い犬（605頭）の糞便を用いた結果で、平均＋3SDを擬陽性カットオフ値、平均＋5SDを陽性カットオフ値とした場合、擬陽性反応率1.8%、偽陽性反応率0%であった。以上の事から、このサンドイッチELISAによりスクリーニングを実施し、感染犬に対する迅速な対応を行い。最終的にはPCRによりエキノコックスDNAの特異的検出もしくは片節検出により確定診断を行っている。

感染犬が発見された時の対応

感染症新法が1999年4月より施行され，エキノコックス症は第4類感染症に含まれるようになり、患者数の全数把握のため、エキノコックス症と診断した場合は医師に届け出義務が課せられるようになった。一方、犬やキツネについての届け出義務はないが、虫卵を排泄するので、人への感染予防の観点からは迅速な届け出が必要であり、新たな改訂が行われる予定である。

前述したように、時折飼い犬においてエキノコックス感染例が発見されるため、北海道小動物獣医師会と北大寄生虫学教室の共同でエキノコックス対応マニュアルを作成した(4)。犬へのプラジカンテル投与によりほぼ完全に駆虫されるが、虫卵を排泄するめため、注意が必要であり、住民のその後の定期的な血清検査が必要となる。

キーワード：多包条虫、飼い犬、診断、対策、ガイドライン

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札幌市北東部における多包条虫媒介動物調査

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Epizootiology of *Echinococcus multilocularis* in the northeastern region of Sapporo

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多包条虫は人に多包虫症を引き起こす人獣共通寄生虫で、その自然界における生活環は終宿主のキツネと中間宿主の野ネズミにより構成される。北海道ではキツネにおける感染率の増加、キツネのヒト生活圏への侵入、飼いイヌ・ネコへの感染などにより、ヒトへの感染リスクが増加することが危惧されている。本研究は今後の都市キツネに対する対策のための基礎データを得るため、札幌市辺縁部の多包条虫媒介動物における多包条虫流行の現状を明らかにし、媒介動物の生息する環境を調べた。札幌市の東北部は川や市街地により周辺からのキツネの侵入が制限されており、その地域内で繁殖・生息するキツネの多包条虫の動物疫学を解明するためには適した地域である。

本研究では、札幌市北東部およびその周辺におけるキツネの活動の拠点としての営巣地の位置を特定し、多包条虫の流行状況を知るためにキツネを捕獲・剖検を行なった。さらに、これらのキツネの生息地において採集したキツネの糞便を用いて糞便内抗原と虫卵検査を行ない、流行状況解明の一助とした。さらに野ネズミからの多包条虫伝播の機会を知るため、営巣地の周辺におけるエゾヤチネズミの分布を調べ、多包虫感染状況を調査した。

2003年５月から９月まで、札幌市北東部およびその周辺（江別市、当別町）において捕獲された25頭のキツネを剖検した。これらは捕獲場所から9グループに分けられた。当別の畑、川岸の荒地、および山間地では3グループ（G-I）、７頭捕獲されたが、感染ギツネは発見されなかった。札幌市北東部（A, C-F）と江別市（C）では６グループ（A-F）、18頭が捕獲された。このうち4グループ（A, B, C, E）、６頭（感染率33％）から感染ギツネが発見された。調査地域ではキツネ営巣地を3ヶ所発見し、それらは牧草地にある未使用の倉庫の床下、畑地内の廃屋および小さな沼と川に挟まれた小さな林にあった。キツネ捕獲地・営巣地は川岸、畑地、山間地で、営巣地または捕獲地から最も近い民家までの距離は0.08～0.8km、民家の密集している住宅地までの距離は0.7～2.3kmであった。捕獲地域周辺の畑、倉庫、道路沿いでキツネの糞便（15個）が採集され、抗原および虫卵陽性糞便（２個）が見つかった。このうちの１個の陽性糞便は貢献において感染ギツネがみつからなかったグループ（F）の活動地域で発見されたものであった。キツネ営巣地周辺においてエゾヤチネズミの生息（延べトラップ数320、８ヶ所）調査を行ない、畑の端、荒地の笹叢や雑草地からエゾヤチネズミ16匹が（６ヶ所／８ヶ所）捕獲されたが、多包虫に感染したエゾヤチネズミは発見されなかった。以上のように札幌北東部で、多包条虫感染ギツネとエゾヤチネズミの生息が確認され、多包条虫がこの地域内で定着していることが示唆された。

今回調査を行なった札幌市の東北部は地理的に隣接した地域から河川で隔離されて、キツネの行動範囲はこの地域内に制限されていると考えられる。したがって、札幌市北東部は駆虫薬入りのベイトを撒くことにより、効率的にキツネの多包条虫の感染率を低下させ、市民の健康を守ることができると考えられた。

Key words: *Echinococcus*, Urban, fox

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テニア科条虫卵の同定法、特に虫卵DNAの抽出とCO I遺伝子の利用

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Identification of taeniid cestode eggs - extraction of egg DNA and use of CO I gene

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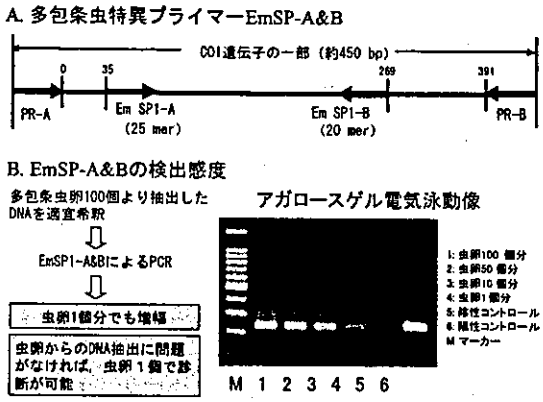
エキノコックスを含むテニア科条虫は主に食肉類を終宿主とするが、糞便検査において虫卵の形態から種を同定することは困難である。我々は、糞便内抗原および虫卵の検出によりエキノコックスの終宿主診断を行っているが、その補足診断法として、虫卵DNAの利用によるテニア科条虫種の同定法を検討した。

まず、猫条虫卵および豆状条虫卵を用いて糞便材料からの虫卵分離およびDNA抽出法について検討した。虫卵分離については、糞便の蔗糖浮遊液に対するナイロンメッシュ濾過法（ポアサイズ40μmで濾過、20μmで捕捉）が効率的であった。DNA抽出は市販のQIAamp DNA Mini Kit (Qiagen) を用いることで、従来必要であった幼虫被殻の破壊処理（KOH処理、SDS添加後煮沸処理、または凍結・融解処理）を省略することができた。70°C 12時間および−80°C冷凍による殺卵処理虫卵ではDNA抽出材料のPCR（扁形動物共通プライマーPRA, PRBによる増幅）で同程度の増幅が確認できたが、1％ホルマリン固定した虫卵では増幅が認められなかった。

これと平行して、エキノコックス属3種12株（系統または分離株）（多包条虫7株、単包条虫4株、フォーゲル包条虫1株）およびテニア属5種23株（猫条虫12株、胞状条虫3株、豆状条虫2株、肥頭条虫5株、羊条虫1株）、合計35株の虫体を用いてCO I領域の塩基配列を決定した。得られた配列とすでに報告されている各種テニア科条虫種の配列とを比較解析して、多包条虫特異プライマーEmSP1-A & Bを構築した（図A）。各種テニア科条虫の抽出DNAに対してEmSP1-A & Bを用いたPCRを行ったところ、多包条虫でのみ増幅像が得られ、EmSP1-A & Bの種特異性が確認された。このプライマーの感度を評価するため、70°C 12時間加熱した多包条虫卵を用いてPCRを行ったところ、虫卵1個分のDNAテンプレート



で増幅像が確認できた（図B）。また、適当な制限酵素を用いたCOI領域のPCR-RFLPにより、猫条虫（Eag I、Xho I）、胞状条虫（SexA I）、肥頭条虫（Sfc I）、豆状条虫（Nsi I）、羊条虫（Msl I）および多包条虫以外の包条虫3種（EcoR I、Hph I）が同定できる可能性が示された。

Key words: *Echinococcus*, egg, DNA

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関東地方におけるイヌおよびネコの寄生虫疫学調査

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A survey of gastrointestinal parasites in dogs and cats in Kanto district

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感染症法が平成16年11月に改正されたが、これに伴い獣医師その他の獣医療関係者は、感染症の予防に関し国及び地方公共団体が講ずる施策に協力するとともに、その予防に寄与するよう努めなければならない、と記載されており、獣医師による届け出義務に関しても記載されている。北海道で問題となっているエキノコックス症も四類感染症に指定されているが、北海道以外への感染拡大が懸念されており、イヌやネコが終宿主になり得るなどの点を考慮すると、今後本州の開業獣医師がエキノコックス症を確認する可能性がある。そこで我々は、本州の動物病院に来院したイヌおよびネコの糞便を用いて、エキノコックスを含む消化管内寄生虫の首都圏における感染状況を調査した。

埼玉、東京、千葉および群馬県の動物病院19ヶ所に来院したイヌおよびネコの糞便を回収し、比重1.27のショ糖液を用いて遠心浮遊法にて糞便検査を実施した。エキノコックス感染の確定診断は多包条虫に特異的な抗体を用いたサンドイッチELISA法にて糞便内抗原検査を実施した。さらには、飼い主へのアンケート調査も併せて実施した。

検査結果は図1に示した通り、イヌでは279検体中24検体、8.6%から、一方のネコは96検体中5検体、5.2%から寄生虫卵あるいはシストが確認された。なおELISA法によるエキノコックスの糞便内抗原検出は全ての検体で陰性であった。また、今回の調査の目的の1つであるエキノコックスに関するアンケート結果は図2の通りであるが、飼いイヌあるいはネコがネズミを捕食するのを目撃した事があるか質問したところ、共に90%程度が目撃した事がないとの回答であった。また、北海道あるいは海外への渡航歴の有無に関しては、今回の調査ではイヌ、ネコそれぞれ5頭と1頭、およびイヌ2頭のみが渡航歴があった。今回の調査ではエキノコックス感染は認められなかったが、エキノコックスに感染したイヌが年間300-400頭余り、北海道外へ移動しており、エキノコックス汚染国からも数百頭のイヌが無検疫のまま輸入されている、との土井らの報告があることなどから、今後もエキノコックスモニタリングを継続すると共に、調査範囲を拡大する必要があると考えられる。

図1　動物病院に来診したイヌおよびネコの糞便検査結果

動物種	頭数	陽性数	陽性率(%)
イヌ	279	24	8.6
ネコ	96	5	5.2
不明	10	0	0
	385	29	7.5

<イヌ>

感染寄生虫種	陽性数	陽性率(%)
イヌ鞭虫	15*	5.4
Isospora spp.	4	1.4
イヌ回虫	3*	1.1
イヌ鉤虫	3	1.1
	25*	8.6

*重感染個体1頭を含む

<ネコ>

感染寄生虫種	陽性数	陽性率(%)
ネコ回虫	5	5.2

ELISA法による多包条虫糞便内抗原の検出は全ての検体で陰性

図2　糞便検査とアンケート結果

<イヌ>

ネズミ捕食行動目撃	頭数
ある	3
ない	254
不明	0
未記入	22
	279

北海道渡航歴	頭数
あり	5
なし	229
不明あるいは未記入	45
	279

海外渡航歴	頭数
あり	2
なし	241
不明あるいは未記入	36
	279

<ネコ>

ネズミ捕食行動目撃	頭数
ある	5
ない	84
不明	0
未記入	7
	96

北海道渡航歴	頭数
あり	1
なし	80
不明あるいは未記入	15
	96

海外渡航歴	頭数
あり	0
なし	86
不明あるいは未記入	10
	96

Key words: *Echinococcus*, dog, cat

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Short Report

Transmission Electron Microscopy of *Schistosoma mansoni* Cercariae Treated with Hinokitiol (β-thujaplicin), a Compound for Potential Skin Application against Cercarial Penetration

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CHISTY, M.M., NARGIS, M., INABA, T., ISHITA, K., OSANAI, A. and KAMIYA, H. *Transmission Electron Microscopy of **Schistosoma mansoni** Cercariae Treated with Hinokitiol (β-thujaplicin), a compound for Potential Skin Application against Cercarial Penetration.* Tohoku J. Exp. Med., 2004, **202** (1), 63-67 —— Since skin is the only route of entry of the parasite in schistosomiasis patients, intervention at the level of skin penetration should control the infection. Several compounds were screened for their ability to protect against cercarial penetration. Hinokitiol (β-thujaplicin) was found to have a significant cercaricidal effect in vitro, although there is no information on its cercaricidal mechanisms. To study the kinetics of morphological changes in *Schistosoma mansoni* associated with exposure to hinokitiol in vitro, cercariae were incubated in media containing hinokitiol at different concentrations and examined by transmission electron microscopy (TEM). TEM revealed that ultrastructural changes occurred by 15 minutes post exposure, at a concentration of 25 μg/ml. Degenerative changes involving both tegument and deeper parenchymal structures were progressive with duration of exposure at the concentration of 50 μg/ml. These structural changes may account for the inability of hinokitiol-treated cercariae to infect the host. ——— hinokitiol; *Schistosoma mansoni*; cercariae; transmission electron microscopy

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Schistosomiasis is a life threatening tropical disease of humans. Non-immune travelers visiting endemic areas are at high risk of acquiring this disease and disseminating the parasite to non-endemic areas (Stone 1995). Since skin is the only route of entry for this parasite into humans, intervention that prevents entry of cercariae into the skin should control the infection (Stirewalt and Dorsey 1974). Several compounds were screened for their ability to confer protection against cercarial penetration following skin application. Among these, a few compounds (diethyl boramide, niclosamide, cederol, β-thujaplicin and diethyl toluamide) showed significant cercaricidal effects in vitro (Naples et al. 1992; Abu-Elyazeed et al. 1993; Nargis et al. 1997; Salafsky et al. 1998). β-thujaplicin is a tropolone-related compound found in the heartwood of several cupressaceous plants such as western red cedar (*Thuja plicata*), eastern white cedar (*Thuja occidentalis*) and hinoki cypress (*Chamaecyprais obtusa*) (Nozoe 1936; Erdtmann and Gripenberg 1948). It is known to be effective as an antimicrobial agent (Katsumura et al. 1948; Kobori and Tanabe 1993, 1994), and is being used as an anti-bacterial hand washing solution (Kobori and Tanabe 1994). However, a review of literature reveals no ultrastructural information of hinokitiol-treated cercariae. The aim of this study, therefore, was to evaluate the in vitro effects of hinokitiol on cercariae of *Schistosoma mansoni* at the level of ultrastructure.

Materials and Methods

A Puerto Rican strain of *S. mansoni* was maintained in our laboratory by passage through Mongolian gerbils, *Meriones unguiculatus*, and *Biomphalaria glabrata*. Cercariae were obtained from the infected snails and used for experiments within 1 hour of shedding.

Synthesized hinokitiol powder of 100% purity was obtained from Takasago International Co. Tokyo. Hinokitiol powder dissolved (200 μg/ml) in water and serial dilutions were prepared from 25 to 50 μg/ml in distilled water (Nargis et al. 1997). Four ml of each dilution was transferred to each culture tube. Control tubes received an equal amount of water. Approximately 400 cercariae of *S. mansoni* were placed in each test tube. Samples were collected at 5, 15, 30, 60 and 120 minures after exposure to hinokitiol at each concentration and processed for transmission electron microscopy (TEM).

Cercariae collected from each tube were transferred to a fresh tube containing 10 ml of RPMI 1640 medium plus 10% foetal calf serum (JRH Bioscience, Kansas) and concentrated into a pellet by centrifugation at 300 g for 10 minutes. The pellet was washed with the same medium, then fixed overnight at 4°C in a fixative containing 3% glutaraldehyde and 1% formaldehyde in 0.1 M phosphate buffer (PH 7.4). After 1 hour post-fixation in 1% osmium tetraoxide the pellets were dehydrated through a series of ethanol and embedded in epoxy resin. Methylene blue stained sections were examined light microscopically and ultrathin sections were examined in a transmission electron microscope (JEOL, Tokyo) after staining with uranyl acetate and lead citrate.

Results and Discussion

Cercariae exposed to hinokitiol at concentrations of 25 μg/ml or more showed progressive morphological changes (Figs. 1 and 2), observed earliest at 15 minutes post exposure. Gross changes were observed, including cercarial tail loss in about 50% of cercariae at 15 minutes, and 90% by 30 minutes after exposure. These observations were consistent with the report by Nargis et al. (1997), which showed that hinokitiol at the concentration of 25 μg/ml significantly affected the cercarial movement and swimming activity. Epon embedded thin sections were observed light microscopically and showed progressive degeneration of the acetabular glands (Fig. 1C), thinning of the tegument causing external protrusion with focal loss of spine (Figs. 1D and 1E), edematous swellings and other degenerative changes (Fig. 1F). Control cercariae exhibited normal morphology (Figs. 1A and 1B).

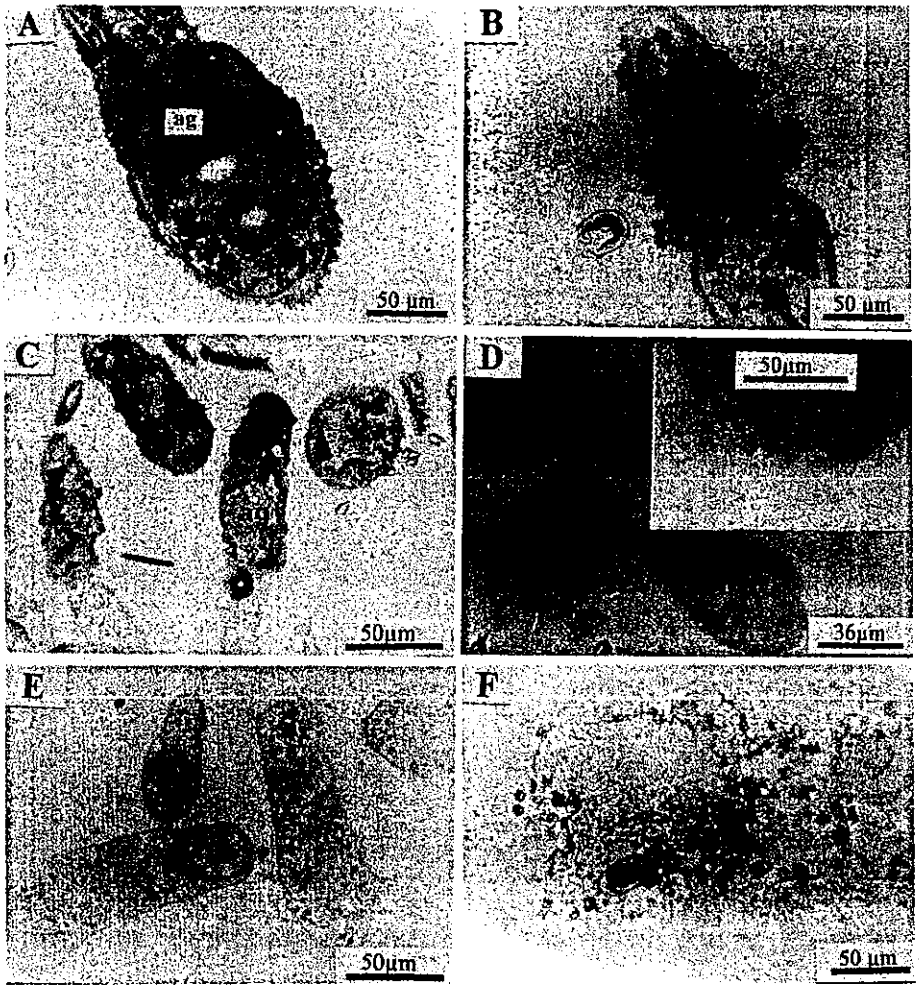

Fig. 1. Kinetics of morphological changes observed in cercariae of *Schistosoma mansoni* treated with hinokitiol (25 µg/ml).
A) Control cercaria at 30 minutes; B) Hinokitiol-treated cercariae at 5 minutes; C) at 15 minutes, partial degeneration of the acetabular glands; D) at 30 minutes, complete degeneration of the acetabular glands with external protrusion (arrow head, inset); E) at 60 minutes; F) at 120 minutes post exposure, severe edema leading to rupture of the cercariae. ag, acetabular glands. Epon embedded Methylene blue stain.

Ultrastructural changes were evident as early as 15 minutes post exposure to hinokitiol, and became more severe with increased duration of exposure. Early degenerative changes included accumulation of membranous bodies (Fig. 2B), migration of cytoplasmic granules into the tegument (Fig. 2C) and loss of external glycocalyx resulting extreme thinning of the tegument (Figs. 2C, 2D and 2E), diffuse edematous changes in the parenchyma and focal lysis of the tegument causing expulsion of sub-tegumental materials (Fig. 2D). These changes were noted in all of the parasites examined, although to a varying degree. Changes progressed in severity with increased duration of exposure (Figs. 2E and 2F).

Topically applied hinokitiol may not efficiently diffuse into the host skin and nor alter the host immune response against migrating schistosomes, although in vitro experiments suggested an immunosuppressive effect (Inamori et al. 1993). This study demonstrated that hinokitiol has a damaging effect in vitro on *S. mansoni*

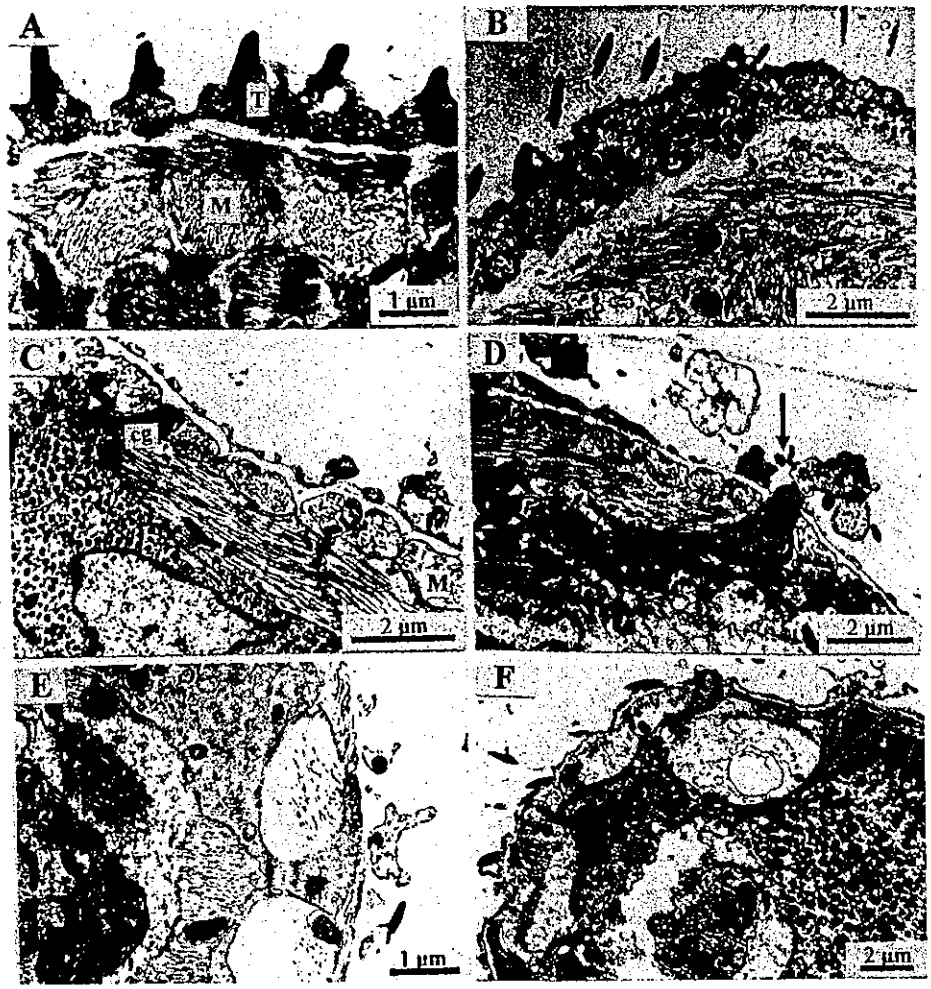

Fig. 2. Kinetics of ultrastructural changes in the cercariae of *Schistosoma mansoni* exposed to hinokitiol (25 µg/ml). A) at 120 minutesutes, Control cercaria, showing intact corrugated tegument (T), compact muscle layer (M) without cellular swelling or edema; B) at 15 minutes, hinokitiol-treated cercariae accumulation of membranous bodies in the tegument; C&D) at 30 minutes, migration of cytoplasmic granules (cg) towards the tegument and thinning of the tegument with degenerative changes causing focal breach of continuity of the tegument (arrow); E) at 60 minutes, edematous swelling of the muscle layer and deeper parenchyma; F) at 120 minutes, severe degeneration leading to membrane rupture.

cercariae in a concentration and time dependent manner. The minimum concentration (25 µg/ml) required to produce demonstrable degenerative changes (Figs. 1 and 2) is lower than the concentrations reported for a variety of bacteria and fungi, where effective concentrations of hinokitiol were 100 or 50 µg/ml (Kobori and Tanabe 1993; Okabe et al. 1989). In previous experiments we have reported that hinokitiol (50 µg/ml) treated-cercariae showed defective motility and infectivity (<1% adult worm recovery in mice vs. >60% in vehicle controls (Nargis et al. 1997). Based on the results from the present study, degenerative structural alterations prevented the cercariae from penetrating the host skin.

Apart from anti-microbial effects, various bioactivities of hinokitiol have been reported, such as repellent activity for ticks, inhibitory effect on the germination of plant seeds and the growth of roots of several plants, cytotoxic effect on tumor cells, and lymphocyte blastogenesis (Okabe et al. 1988; Inomori et al. 1991, 1993).

The mechanism underlying broad range bioactivities and the target(s) is still unrevealed. In vitro cytotoxic and immunosuppressive effects of hinokitiol have been reported; namely it prevents ultraviolet radiation-induced apoptosis in keratinocytes of mice (Baba et al. 1998), and influenza virus-induced apoptosis, replication and release from the infected Madin-Darby canine kidney cells (Miyamoto et al. 1998).

Hinokitiol is generally considered safe to host skin even at high concentrations, as it might not be absorbed through the host skin (Nargis et al. 1997). Furthermore, hinokitiol applied to the skin did not alter the migration of epidermal Langerhans' cells to regional lymph node of guinea pigs infected with *S. mansoni* (Chisty, M.M. et al.; unpublished data). Intervention may be achieved by application of hinokitiol with an appropriate base that may help it persist on the skin surface and make it more resistant to removal by contact with water.

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SCHISTOSOMA MANSONI: KINETICS OF GLOMERULONEPHRITIS IN MONGOLIAN GERBILS AND ITS CORRELATION WITH INTENSITY AND DURATION OF INFECTION

CHISTY M.M.*, NARGIS M.*, SATO H.*, INABA T.*, TAKAHASHI G.** & KAMIYA H.*

Summary:

The frequent occurrence of glomerular lesions in schistosomiasis patients has been reported, although appropriate animal models for the study of schistosomal glomerulonephritis have not been developed. To analyze the relationship between glomerulonephritis and *Schistosoma mansoni* infection, gerbils, *Meriones unguiculatus*, were infected with different number of cercariae and sacrificed at different weeks of post infection. Fifty cercariae were the optimum dose to produce the disease, glomerulonephritis, without early death of the animal. Infected gerbils showed heterogeneous types of glomerular lesions with increased serum creatinine level. Immune complex deposition was not detected at glomeruli of infected gerbils even by means of immunuofluorescence and also by transmission electron microscopy. However, infiltration of mononuclear cells in and around some of the altered glomeruli was observed. Immunohistochemical staining, using monoclonal antibody (HUSM-M.g.15) specific to gerbil's T-cells, revealed significant infiltration of T-cells. These findings suggest that T-cells might be involved in the development of glomerulonephritis. Gerbil could be a useful model to clarify the role of T-cells in the development of glomerulonephritis of schistosomiasis.

KEY WORDS : glomerulonephritis, *Schistosoma mansoni*, Mongolian gerbils, T-cells, immunohistochemistry.

Résumé : *SCHISTOSOMA MANSONI* : DÉVELOPPEMENT D'UNE GLOMÉRULONÉPHRITE CHEZ LA GERBILLE DE MONGOLIE ET CORRÉLATION AVEC L'INTENSITÉ ET LA DURÉE DE L'INFECTION

Les lésions glomérulaires des patients atteints de schistosomiase ont été souvent rapportées, cependant, il n'a pas été développé de modèle animal permettant d'étudier les lésions de glomérulonéphrites. Afin d'analyser les relations entre glomérulonéphrite et infection à Schistosoma mansoni, *des gerbilles,* Meriones unguiculatus, *ont été infectées avec des quantités variables de cercaires et sacrifiées à des dates différentes après cette infestation. 50 cercaires sont la quantité optimale pour provoquer une glomérulonéphrite sans entraîner la mort de l'animal. Les gerbilles infectées montrent des lésions glomérulaires hétérogènes avec une élévation de la créatinine sérique. Il n'a pas été observé de dépots de complexes immuns au niveau des glomérules, ni en immunofluorescence, ni en microscopie électronique à transmission. Cependant, un infiltrat de cellules mononuclées dans et autour certains glomérules a été observé. Une étude immunohistochimique, utilisant un anticorps monoclonal (HUSM-M.g.15) spécifique de cellules T de gerbilles, a révélé une infiltration de ces cellules. Cette découverte suggère que les cellules T pourraient intervenir dans le développement de la glomérulonéphrite. Les gerbilles pourraient être un bon modèle afin de clarifier le rôle des cellules T dans le développement des glomérulonéphrites schistosomiennes.*

MOTS CLÉS : *glomérulonéphrite,* Schistosoma mansoni, *gerbille, cellules T, immunohistochimie.*

INTRODUCTION

Infection of humans with schistosomes causes schistosomiasis, affects approximately 300 million peoples, is the most important cause of glomerulonephritis among parasitic infections in Africa, and Latin America (reviewed by Barsoum, 1993). Glomerulonephritis may be defined as a pathological process, characterized by focal or diffuse proliferation, infiltration or destruction of the glomerulus with or without involvement of the tubules or interstitial tissues. The incidence of such glomerulonephritis among patients with *S. mansoni* hepatosplenic disease was variably reported from 15 to 40 % (Andrade *et al.*, 1971; Rocha *et al.*, 1976). It was thought that schistosomal glomerulonephritis might be a typical example of immune complex (IC) glomerulonephritis (GN) because of their presence of schistosomal worm antigen (De Brito *et al.*, 1998) and IC in the glomeruli (Sobh *et al.*, 1991). However, treatment did not show any improvement rather progression to chronic renal failure (Sobh *et al.*, 1988), and polyclonal B-cells activation alone was not enough to induce GN in mice (Fujiwara *et al.*, 1988). Thus besides IC, host related factors, such as T-cells or macrophage function, seem to be involved (Van Velthuysen, 1996). Several laboratory animal species from mouse to chimpanzee have been used for the study of *S. mansoni* infection (De Brito *et al.*, 1971; Brack *et al.*, 1972; Andrade & Susin, 1974; Sobh *et al.*, 1991). However as a model of the disease, none of these hosts was considered as ideal. For example, the lesions in chimpanzees closely resemble those in humans (Sadun *et al.*, 1975),

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but these animals are not widely accepted to use for animal experiments. On the other hand, hamsters and mice spontaneously develop renal pathology with age (Robinson *et al.*, 1982). An animal model should provide a normal worm development and the lesions comparable to those of human. In addition, it should be easily maintained. Worm development and liver lesions recorded in gerbils infected with *S. japonicum* more closely resembled to those of humans than did the lesions observed in mice or rabbits (Yingrui *et al.*, 1983). But the literature does not contain any information regarding schistosomal glomerulonephritis in gerbils.
The objective of this experiment was to study the glomerulonephritis in gerbils infected with *S. mansoni* and its correlation with intensity and duration of infection.

MATERIALS AND METHODS

PARASITES

A Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* snails and Mongolian gerbils, *Meriones unguiculatus*, was used through out the experiments. Cercariae were used within one hour of being shed.

ANIMALS

In the present study 180 gerbils, 6-8 weeks old of either sex were used. Among these, 144 were infected with different doses of cercariae (25, 50, 100 and 150 cercariae) of *S. mansoni* and the remaining 36 animals served as controls. These animals were bred at the Institute for Animal Experiments of our university. Animals were fed food pellets and water *ad libitum*. All animal experiments were performed according to the Guidelines on Animal Experimentation as set out by Hirosaki University.

INFECTIONS

The animals were anaesthetized by intraperitoneal injection of 30 mg/kg Nembutal® (Pentobarbital sodium; Abbot Laboratories, North Chicago, USA). The infections with cercariae were carried out by the ring method of Smithers and Terry (1965). The mean number of cercariae used in each animal was calculated from six random aliquots of the cercarial suspension. For cercarial penetration, one-hour was allowed after which the water in the ring was examined for non-penetrating cercariae.

LABORATORY EVALUATIONS

S. mansoni infected gerbils and controls matched for age and sex were subjected to the following measurements: 1) Serum creatinine concentration (mg/dl); 2) blood urea nitrogen (BUN) concentration (mg/dl); 3) Serum albumin, globulin and total protein concentration (mg/dl), and serum cholesterol concentration (mg/dl). Automatic Biochemical Analyzer (Olympus AU 600) was used for biochemical evaluations.

HISTOPATHOLOGICAL EVALUATIONS

Animals were killed by an anesthetic over dose of ether, at various weeks (wks) post infection (p.i). Kidney tissues of the sacrificed animals were subjected to the following examinations:

A) Light microscopic examination

All collected kidney samples were fixed in 10 % neutral phosphate buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H/E), Periodic Acid Schiff (PAS), Periodic acid silver methanamine (PASM) and Congo red stains. In average, four sections were made from the hilar region of each kidney and were examined microscopically. In each animal, 30 glomeruli were randomly selected from four sections. The mean number of cells per glomerular cross-section (c/gcs) was counted by using high power objectives. The mean glomerular diameter was measured by means of an ocular micrometer. Glomerular abnormalities, especially mesangial cell proliferation, alteration of the mesangial matrix, thickening of the glomerular basement membrane (GBM), hemorrhage and necrosis along with tubulo-interstitial changes were recorded.

B) Transmission Electron Microscopy (TEM)

TEM was performed in three animals from each group at each time points. Removed kidneys were immediately sliced at 0.5 mm thickness, and prefixed in cold 2.5 % glutaraldehyde solution in 0.1 M phosphate buffer (PB), pH 7.4, at 4° C for more than two hours. They were washed in two changes of cold PB for 10 min, and post-fixed in cold 1 % osmium tetra-oxide in PB for two hours. Specimens were then washed in three changes of cold distilled water, stained *en block* with 1 % uranyl acetate, dehydrated in a series of alcohol, and embedded in epoxy resin. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed with an electron microscope (JEOL, Japan).

C) Immunohistochemical examination

a) Immunofluorescent microscopy for detection of immune complex-related immunoglobulins (IgG, IgM, and IgA): Kidney cryostat sections (5 µm thick) were air-dried and fixed in acetone for 10 min. The sections were washed with phosphate buffered saline (PBS), pH 7.3 and incubated with PBS containing 10 % normal goat sera to block non-specific binding sites. Indirect immunofluorescence techniques were applied using a panel of antibodies cross-reactive with gerbils immu-

noglobulins, directed against IgG (rabbit antibody to rat IgG (H + L) (Chemicon International Inc., Temecula, CA, USA); IgM (goat $F(ab')_2$ fragment to mouse IgM) (American Qualex, La Mirada, CA, USA), rabbit IgG to goat IgG (H + L) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and IgA (rabbit antibody to mouse IgA) (ZYMED Laboratories, Inc., San Francisco, CA, USA). Antibodies were applied in a working dilution 1:100 as first layers. FITC-conjugated affinity purified goat anti-rabbit IgG (E.Y Laboratories, Inc., San Mateo, CA, USA) were used as second layer in a working dilution 1:200. For control sections the primary antibody was omitted to assess non-specific staining. Kidney cryostat sections from normal gerbils were used as negative control.

b) Immunohistochemical staining for visualization of gerbil T-cells: Cryostat sections were air dried and fixed in cold acetone for 10 min. The immunohistochemical staining was performed using a novel mouse monoclonal antibody (HUSM-M.g.15 of IgG2b isotype) specific to gerbil T-cells (Sato *et al.*, 2000). Undiluted culture supernatant was applied as first layer. Peroxidase conjugated goat $F(ab')_2$ fragment to mouse IgG (Fc) (Organon Teknika Corp., Durham, NC, USA) was used as a second layer in a working dilution of 1:200. Bound antibody was detected using color development by 3, 3'-diaminobenzidine, followed by light counter staining with hematoxylin. Intraglomerular and interstitial T-cells infiltration were estimated in 30 glomerular cross-section (gcs) and 50 high power fields (HPF) for each animal, respectively.

STATISTICAL ANALYSIS

Statistical significance of the results was determined using Student's *t*-test. Data were expressed as mean ± SD and a *P* value of less than 0.05 was taken as the minimum level of significance.

RESULTS

Gerbils showed glomerulonephritis, 17 % (25/144) of the total infected, earliest at 20 wk post infection (p.i). However, the prevalence of such glomerulonephritis became more than 80 % in the group infected with 50 cercariae or more at 30 wk p.i (Fig. 1A). In this study animals were defined positive when over 40 % of the glomeruli present in three non-consecutive kidney sections showed histological and immunopathological lesions. Fifty cercariae were the optimum dose to produce glomerulonephritis without early death of the animals. It was confirmed by repeated experiment for its reproducibility (unpublished data). Groups infected with a higher dose of cercariae showed a higher prevalence of glomerulonephritis but a shorter period of survival (Fig. 1A, B). None of the control animals revealed any glomerulonephritis.

Gerbils infected with 50 cercariae showed gradual and consistent elevations of serum creatinine level (Fig. 2A). However, their serum cholesterol and BUN (Fig. 2B) levels were mild and irregular, did not correlate well with the intensity and duration of infection. The increase in total proteins was considerably greater in those infected gerbils while proportional decrease in serum albumin levels was observed in these animals, but the absolute amount of serum albumin did not diminish (Fig. 3A, B).

Mean glomerular diameters gradually increased in the infected groups and became significantly different from those of controls at 20 wk p.i (Table I). Glomerular cell counts gradually increased in all the infected gerbils. The increased cellularity became significant from 20 wk p.i in all the groups infected with 50 cercariae and more (Table II). Glomerular hypercellularity was due to infiltration of inflammatory cells and endocapillary cellular

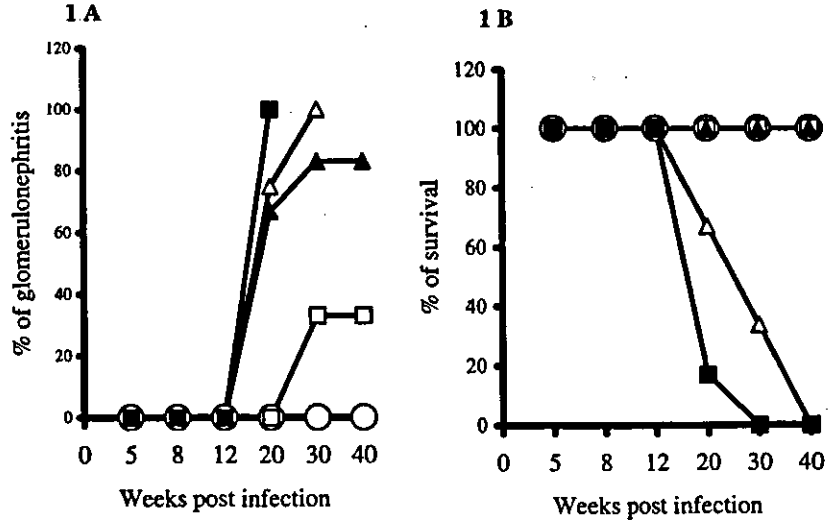

Fig. 1. – Effect of intensity and duration of infection with *Schistosoma mansoni* cercariae on the prevalence of glomerulonephritis (A) and survival rate of gerbils (B). Gr-1: 0 cercaria (○); Gr-2: 25 cercariae (□); Gr-3: 50 cercariae (▲); Gr-4: 100 cercariae (Δ) and Gr-5: 150 cercariae infection (■). The number of gerbils at the beginning of the experiment was six at each time points.

No. of cercariae infected	No. of cells/glomerulus at different weeks p.i.					
	5 wk	8 wk	12 wk	20 wk	30 wk	40 wk
0	34 ± 1	33 ± 4	34 ± 5	33 ± 3	32 ± 2	36 ± 3
25	37 ± 5	38 ± 6	37 ± 3	38 ± 8	49 ± 4***	57 ± 7***
50	36 ± 4	40 ± 8	40 ± 6	44 ± 5***	54 ± 5***	72 ± 9***
100	34 ± 3	39 ± 8	41 ± 6	47 ± 6[a] **	53 ± 2[b] ***	NA
150	35 ± 3	40 ± 8	40 ± 5	54[c]	NA	NA

Each point represents no. of cells/glomerular cross-section (mean ± SD). Total 30 glomeruli/animal were examined and their contained cells were counted using high power objectives. Cellularity was evaluated by Student's *t*-test and found significant, where $P < 0.05$, comparing with the control (0 cercariae). NA: Animal not available for examination. Six gerbils, at each group except three, where [a]: 4; [b]: 2 and [c]: 1 gerbil. **: $P < 0.01$; ***: $P < 0.001$.

Table II. – Glomerular cellularity of gerbils infected with different number of *Schistosoma mansoni* cercariae at different weeks p.i.

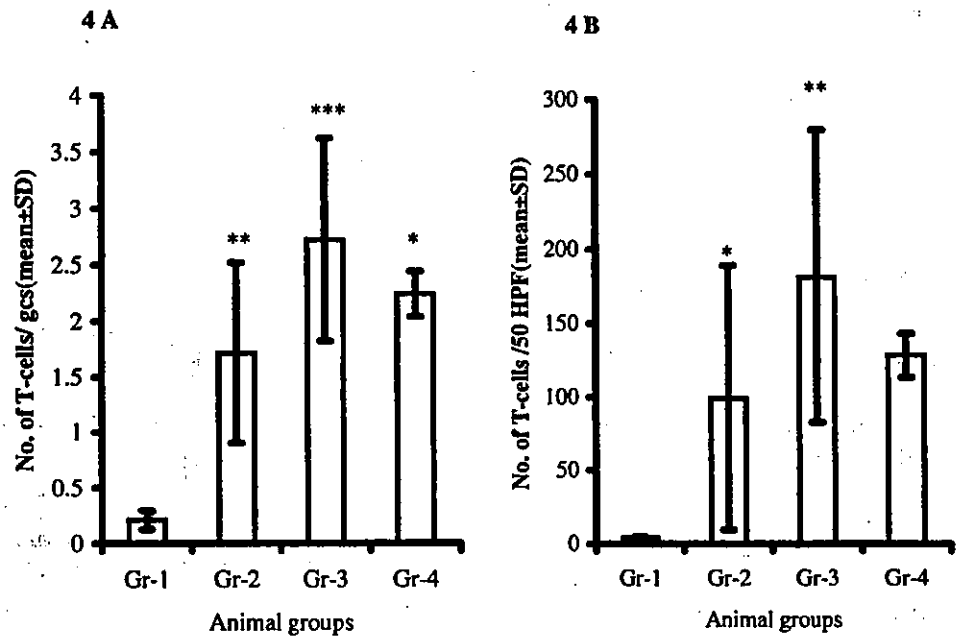

Fig. 4. – T-cell infiltration, intraglomerular (A) and interstitial (B), in gerbils infected with *S. mansoni* at 30-wk p.i. Gr-1: Control, n = 6; Gr-2: infected with 25 cercariae, n = 6; Gr-3: infected with 50 cercariae, n = 6 and Gr-4: infected with 100 cercariae, n = 2. (A) No. of T-cells/glomerular cross-section (c/gcs), total 30 glomeruli/animal were examined; (B) interstitial infiltration of T-cells/50 HPF. The values were evaluated by *t*-test and found significant, where $P < 0.05$, comparing with control group. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

assumed hyaline like appearance in H/E, which was PAS and PASM positive. Some of the tubules showed cellular and hyaline cast. Accumulations of MNCs in and around some of the altered glomeruli (Fig. 5E) were observed. TEM revealed renal abnormalities earliest at 20 wk p.i in the 50 cercariea and more infected gerbils. The abnormalities were wrinkling and irregular thickening of the glomerular basement membrane (GBM) with variable degree of severity (Fig. 6A, B), increased mesangial area and cellularity (Fig. 6C) in more than 40 % of the infected gerbils. Peritubular accumulation of MNCs and tubular basement membrane (TBM) abnormalities were also observed (Fig. 6D). Out of 144 infected gerbils only two showed granuloma with renal egg deposition. Five (3.5 %) of the infected gerbils exhibited amyloid deposition earliest at 30 wk p.i in the renal glomeruli and interstitium. Electron microscopy showed that the glomerular amyloid deposits were mainly subendothelial (Fig. 6E, F). None of the control gerbils showed any amyloid deposits.

DISCUSSION

Schistosomal glomerulonephritis is considered a late complication of hepatosplenic schistosomiasis with collateral circulation, where eggs bypass the hepatic filter and are carried to the lungs and then to the systemic circulation (Andrade *et al.*, 1971). This will permit the diversion of the immune complex (IC) away from the liver and its Kupffer' cells. Thus the complexes will reach the kidney and other organs by the systemic circulation.

In our present study, 50 cercariae were the optimum dose of infection at which majority of gerbils showed glomerulonephritis (Fig. 1A) at 30-wk p.i. This dose was well tolerated up to 40-wk p.i and glomerular changes were almost similar to higher dose groups. None of the control animals showed any glomerulonephritis. The serum biochemical findings in infected gerbils contrasted in some important respects (cholesterol and BUN) with the biochemical observations

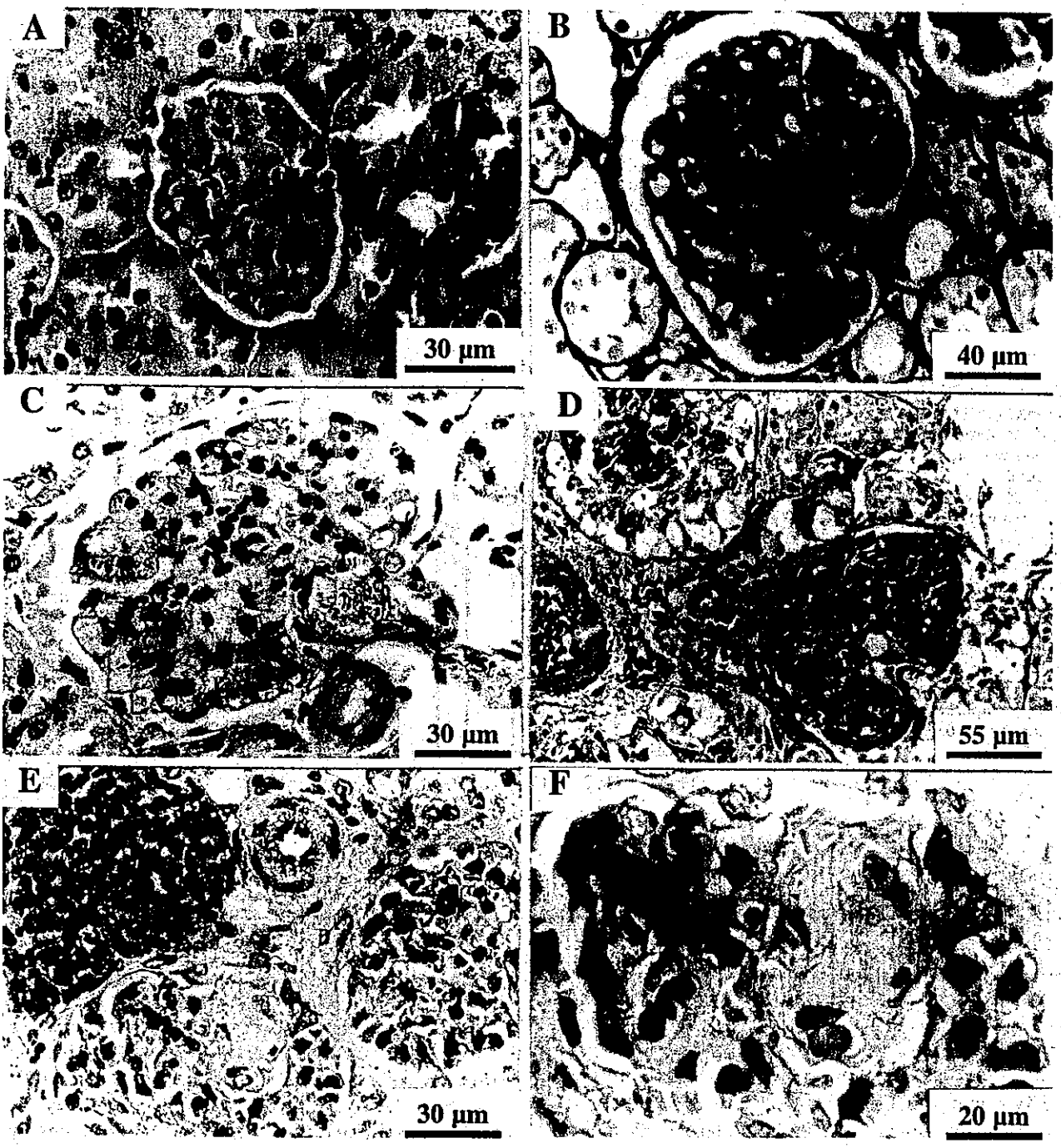

Fig. 5. – Pathological changes in the kidney of gerbils exposed to 50 cercariae at 30 wk p.i. (A) morphologically normal (control, H/E); (B) mesangioproliferative GN (PASM); (C) proliferative GN (H/E); (D) necrotizing GN (H/E); (E) Segmental glomerulosclerosis with periglomerular and interstitial mononuclear cells infiltration (H/E); (F) Glomerulosclerosis where sclerosing capillary loops assumed hyaline appearance (H/E).

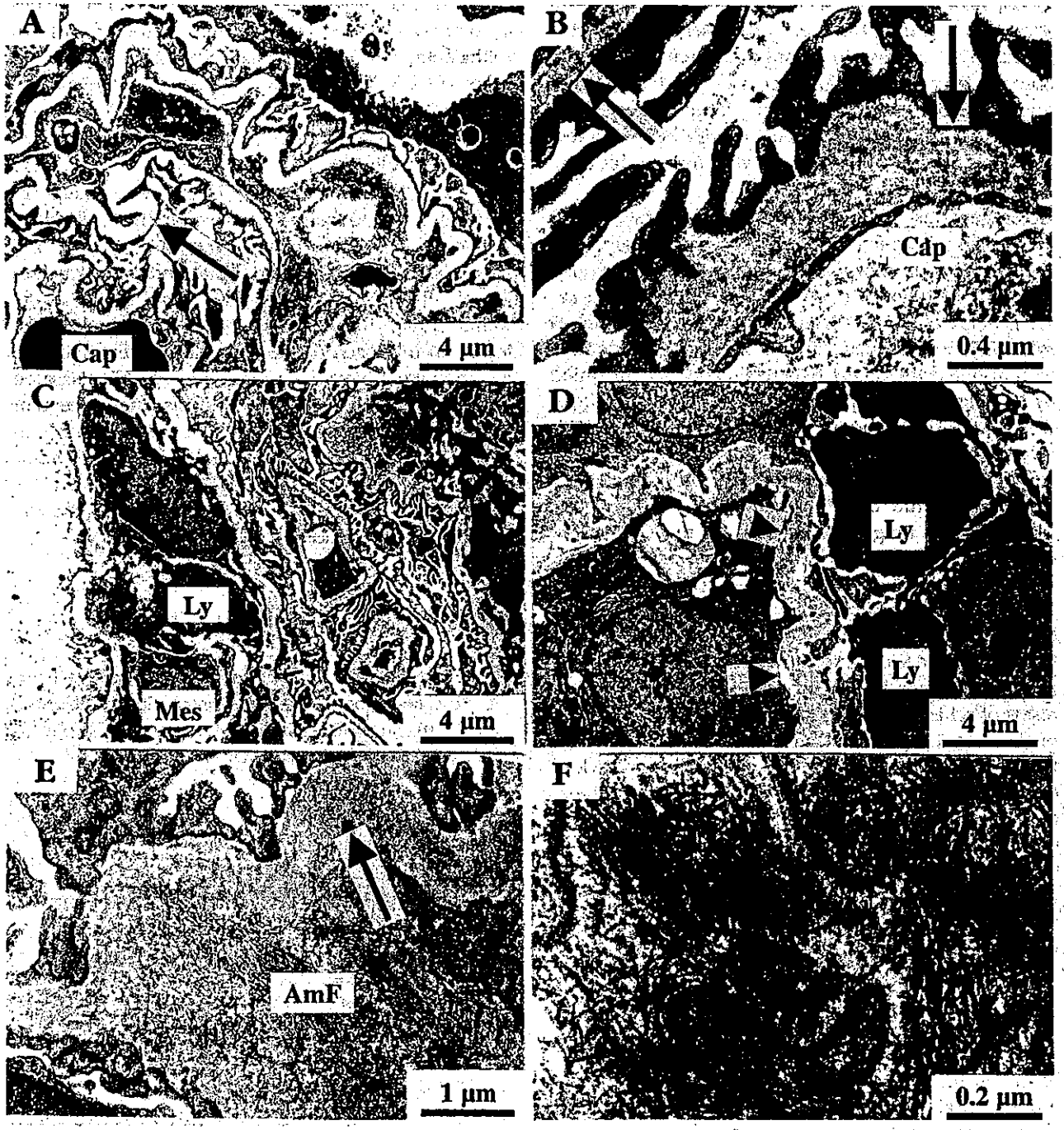

Fig. 6. – Electron micrograph of a glomerulus of gerbils exposed to 50 cercariae of *S. mansoni* is showing wrinkling and irregular thickening of the glomerular basement membrane (A & B) with increased no. of cells in the mesangial area (C), along with alteration of tubular basement membrane (D) at 30 wk p.i., subendothelial amyloid deposition (E) with randomly oriented fibrils of higher magnification (F) at 40 wk p.i. Note: arrow, indicate glomerular basement membrane; arrow head, tubular basement membrane; Mes, mesangial cell; Cap, capillary lumen; Ly, lymphocyte; AmF, amyloid fibril.

reported for mice with schistosomiasis (Sadun & Williams, 1966). On the other hand, the significant and consistent increases in creatinine, total protein and globulin concentration recorded in hamsters (Sobh *et al.*, 1991), mice (Sadun & Williams, 1966) and chimpanzees (Sadun *et al.*, 1970) were also observed in infected gerbils. The increase in globulin concentration in the absence of corresponding increases in albumin produced striking reduced albumin: globulin ratio. These were more evident in the animals with heavier infections and became more marked with time (Sobh *et al.* 1991; Sadun *et al.*, 1970).

Heterogeneous types of glomerular lesions (Fig. 5) along with tubulo-interstitial changes were observed in gerbils. Similar morphological changes were also observed in humans (Cheever, 1968; Andrade *et al.*, 1971; Sobh *et al.*, 1989) and other animals (Sadun *et al.*, 1975; Sobh *et al.*, 1991). Several investigators demonstrated mesangial hypercellularity accompanied by IgG glomerular deposits (Sobh *et al.*, 1991; Hilleyer & Lewert, 1974) without abnormality of the GBM (Hilleyer & Lewert, 1974) in hamsters. Mesangial hypercellularity was seen in our experiment but did not affect all the glomeruli of the infected gerbils. However, wrinkling and irregular thickening of the GBM were observed in more than 40 % of the infected gerbils. But the mechanism of these pathological changes remained unknown. It cannot be explain by renal egg deposition since renal egg depositions were sporadic (2/144) but glomerular lesions were much more prevalent.

Amyloidosis may be one of the pathogenetic mechanisms of schistosomal glomerulonephritis, where 3.5 % of the infected gerbils revealed amyloidosis (Fig. 6E, F). None of the control gerbils showed any amyloid deposits. Amyloidosis secondary to schistosomal infections has also been reported in 8% infected hamsters (Sobh *et al.*, 1991) and 16 % of humans schistosomiasis (Barsoum *et al.*, 1979).

In schistosomiasis, IC mediated glomerulonephritis have been reported in mice (Natali & Cioli, 1976; Fujiwara *et al.*, 1988), hamsters (Sobh *et al.*, 1991), monkeys (Tada *et al.*, 1975) and humans (Sobh *et al.*, 1987). Recently immunoelectron microscopic localization of schistosomal antigen in the glomerulus of hamsters, where mesangial expansion with increased cellularity has been reported (De Brito *et al.*, 1998). It is interesting to note here that GBM, tubules and interstitium were unremarkable in hamsters but in our study, infiltration of MNCs with irregular thickening of the GBM were observed. In our gerbil model the glomerular lesions are most probably not IC mediated, since IF staining and TEM of kidney tissues did not reveal the deposition of IC in the mesangium and capillary walls. In humans IC negative GN with increased number of MNCs in the glomeruli has been reported and subtyping of these MNCs in kidney showed significant increases (0.5 ~ 2 c/gcs) of T-cells (Nolasco *et al.*, 1987; Tipping *et al.*, 1985). There is now evidence that T-cells play a major role in glomerular injury, where $CD4^+$ T-cells responsible for the induction of autoimmune syndrome and glomerular infiltrations of $CD8^+$ T-cells are directly involved in the onset of proteinuria (Van Velthuysen, 1996). Glomerular hypercellularity, due to influx of $CD8^+$ T-cells, was reported in murine malaria (Lloyd *et al.*, 1993).

Significant T-cells infiltrations in and around some of the altered glomeruli were observed in our study (Fig. 4A, B). These infiltrated T-cells were unable to be clarified due to lack of information on T-cell subset of gerbils. This abnormal infiltration of T-cells and macrophages in the glomeruli may indicate participation of cellular immunity (Bolton *et al.*, 1987; Saito & Atkins, 1990; Hooke *et al.*, 1987), although some defects in the macrophage activation system and also in complement have been reported in gerbils (Nasaree *et al.*, 1998; Kamiya *et al.*, 1980). This is in agreement with our previous experiment, where we found lymphocytic myocarditis in gerbils infected with *S. mansoni* (Chisty *et al.*, 1999). To elucidate the role of T-cells in glomerular injury associated with parasitic infections further studies are suggestive.

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Original Paper

Development of *Taenia saginata asiatica* metacestodes in SCID mice and its infectivity in human and alternative definitive hosts

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Abstract Development of *Taenia saginata asiatica* metacestodes in SCID mice, and its infectivity in humans, golden hamsters, and Mongolian gerbils as alternative definitive hosts, were investigated. Cysticerci were recovered from SCID mice that were subcutaneously injected with hatched eggs of *T. s. asiatica*. The morphological changes of cysticerci were observed. The recovered cysticerci were by fed to gerbils, hamsters and humans, to check for infectivity. Tapeworms were recovered from gerbils and hamsters that were fed 20 to 45 week-old cysticerci, and proglottids excretion were observed in human volunteers fed with 45 week-old cysticerci. However, no tapeworms were recovered from gerbils fed with 10 week-old cysticerci. Our results suggest that *T. s. asiatica*