免疫機能制御部門（客員部門）

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本部門では，肝炎の発症と慢性化進行における免疫学的機序を解明し，和漢薬を含む種々の資源から新しい肝炎治療薬の発見などを目的として研究を行っている。主に臨床肝炎の細胞性免疫病理過程を模擬した新しい肝障害動物モデルを用いて，肝障害における細胞及び分子機構，とくに細胞接着およびサイトカインの役割などについて研究を続けている。その上に，和漢生薬およびその活性成分の作用，とくに新しい作用様式に着目し，そのメカニズムの解明を努めている。本年度の主な研究課題と成果は下記の通りである。

Ⅰ．肝障害の発症と進行に関する基礎的研究

1．マウスの肝臓において一回遅延型アレルギー（DTH）反応を引き起こすことによって肝組織障害が数ヵ月も続くことを発見し，種々の生化学的及び病理学的パラメーターを解明した（*Life Sciences* 1997）。このような慢性化持続は従来の皮膚などでのDTH反応と異なって，極めてユニークであり，肝炎慢性化機序の解明に寄与しうると考えられる。さらに，その慢性化過程の進行は$CD4^+$および$CD8^+$T細胞によってそれぞれ正と負の調節をしていることを明かにした（*Int. Arch. Aller. Immunol.* 1998）。

2．この肝障害の発症について，免疫細胞とくに$CD4^+$T細胞が肝臓内に浸潤し，lymphocyte function associated antigen-1（LFA-1）/ intercellular adhesion molecule-1（ICAM-1）interactionを介して肝細胞に障害を与えることを示唆した（*Life Sciences* 1998）。その障害過程においてTh1系cytokineであるIFN-γとIL-2はpro-inflammatory因子として，Th2系のIL-4とIL-5はanti-inflammatory因子として肝細胞の障害に対してそれぞれ正と負の調節役割を果たすことを明かにした（投稿中）。

Ⅱ．DTH 肝障害を薬効評価モデルとしての有用性および肝炎新薬開発の new strategy

DTH肝障害モデルに対する肝細胞庇護薬，免疫調節薬および免疫抑制薬など種々の既知肝炎薬物の影響を検討した。その結果，これらの薬物は各自の特徴をもって有効性を示したので，薬効評価のモデルとして有用であることを証明した（*Pharmacological Research* 1997）。

さらに，DTH 反応各段階に対して選択的に抑制する種々の生薬や漢方方剤がDTH肝障害に対しても有効性を証明したので，肝炎新薬開発のnew strategyとして提唱した（*Pharmacological Research* 1997，和漢医薬学雑誌1998）。

Ⅲ．中薬土茯苓およびその成分 astilbin の薬理活性及び肝障害に対する新しい作用様式

土茯苓の水性エキスおよびastilbinが従来の肝庇護薬と異なった作用様式をもって肝障害改善作用を発見し，そのメカニズムを検討した（投稿中）。

土茯苓の水性エキスはアジュバント関節炎に対する有効性とそのメカニズムを検討した（*Pharmacological Research* 1997）。

Ⅰ. 肝障害の発症と進行に関する基礎的研究

Ⅰ-1. One-shot delayed-type hypersensitivity reaction in the mouse liver causes a sustained liver injury to picryl chloride

The developmental characteristics of liver injury induced by a delayed-type hypersensitivity (DTH) mechanism against picryl chloride were examined for 9 consecutive weeks in 3 mouse strains, BALB/c, Kunming and ICR mice. The changes of most biochemical parameters were similar in these three strains, namely, the activities of serum transaminases, lactic dehydrogenase, and prolidase were elevated significantly on day 1, during the first several weeks, and almost throughout the duration, respectively, of liver injury. The content of liver hydroxyproline was also increased after 1-9 weeks of liver injury. In addition, a significant decrease of liver weight, serum alkaline phosphatase and albumin level was observed in BALB/c and Kunming mice. Similar changes in liver histology were also found in the three strains. The hepatocellular necrosis and inflammatory infiltration into the portal area were the predominant features on day 1 and were still distinct during the subsequent several weeks. The mild or moderate hepatocellular degeneration, regeneration and connective tissue hyperplasia were observed after 1 or 3 weeks. A bridging necrosis between portal and portal was observed in several BALB/c and ICR mice, reflecting the possibility of exacerbation of liver injury. These results suggest that the liver injury could be caused and sustained by a one-shot DTH reaction to picryl chloride. The chronicity of the biochemical and histopathological characteristics may be helpful in elucidating the mechanisms of chronic development of liver injury.

Ⅰ-2. Role of $CD4^+$ and $CD8^+$ T cells in regulating the chronic development of liver injury induced by delayed-type hypersensitivity to picryl chloride

In this study we first investigated the cellular immune responses in mice with chronic liver injury induced by delayed-type hypersensitivity to picryl chloride (PCl). A continuous reduction, after week 3 of liver injury, was observed in the level of PCl-induced contact sensitivity but not in sheep red blood cells-induced footpad reaction, suggesting the presence of PCl-specific suppression. When spleen cells from mice whose liver had been injured for 1 week were systemically transferred into syngeneic recipients with the liver injury, the elevation in serum lactic dehydrogenase and the decrease in alkaline phosphatase and albumin levels in recipient mice were significantly exacerbated. However, when the liver damage in the donor mouse was allowed to proceed for 3, 5 or 7 weeks, above biochemical changes in recipients were recovered to near normal levels. A flow cytometric assay demonstrated that the number of $CD4^+$ T cells in both spleen cells and liver nonparenchymal cells decreased dramatically during the late phase of liver injury, while $CD8^+$ counts did not. These findings suggest that $CD4^+$ and $CD8^+$ T lymphocytes may contribute to the positive and negative regulation, respectively, of the early and late phases in the chronic development of liver injury.

Ⅰ-3. LFA-1/ICAM-1 interaction is essentially involved in the pathogenesis of delayed-type hypersensitivity -induced liver injury to picryl chloride

The kinetics of lymphocyte function associated antigen 1 (LFA-1) expression on spleen cells (SPC) and liver non-parenchymal cells (NPC), and intercellular adhesion molecule 1 (ICAM-1) expression on hepatocytes (HC) was examined in acute liver injury mice induced by a DTH reaction to picryl chloride (PCl). The peak expression of LFA-1 on SPC was seen at 6 hr after eliciting liver injury, and then that of LFA-1 on NPC and ICAM-1 on HC appeared at 12 hr. Thereafter, the serum ALT elevation reached to a peak at 18 hr. A splenectomy before the PCl elicitation significantly reduced the ALT elevation. Both SPC and NPC from liver injury mice induced a remarkable release of ALT from HC *in vitro,* in parallel with their LFA-1 expression. The pretreatment of NPC or SPC with anti-LFA-1 mAb, irrespective of the presence of complement, completely blocked the ALT release. Also, when HC was prebound with anti-ICAM-1 mAb, neither NPC nor SPC showed a cytotoxicity against the HC. Furthermore, the treatment of NPC

with either anti-Thy1.2 or anti-CD4 mAb in the presence but not absence of complement, showed a complete abolishment of ALT release. Anti-CD8 mAb plus complement also tended to inhibit ALT release. The twofold increase in $CD4^+LFA\text{-}1^+$ and mild increase in $CD8^+$ $LFA\text{-}1^+$ populations were also confirmed in NPC at 12 hr. These results suggest that PCl elicitation in liver may trigger an increased expression of LFA-1 on SPC and NPC and ICAM-1 on HC. LFA-1/ICAM-1 interaction between liver-infiltrating NPC, mainly including $CD4^+$ and $CD8^+$ T cells, and HC may be an essential step for the hepatocyte damage in PCl-DTH liver injury. HC reached a peak 12 hr after the eliciting. After the peak, the expressions of both LFA-1 and ICAM-1 were reduced and maintained in a level slightly higher than that on 0 hr. The serum ALT activity significantly elevated at 12 hr and reached a peak at 18 hr after the challenge. These findings indicate that the increased expression in LFA-1 on SPC and NPC as well as in ICAM-1 on HC may be linked to the production of liver injury (Fig. 1).

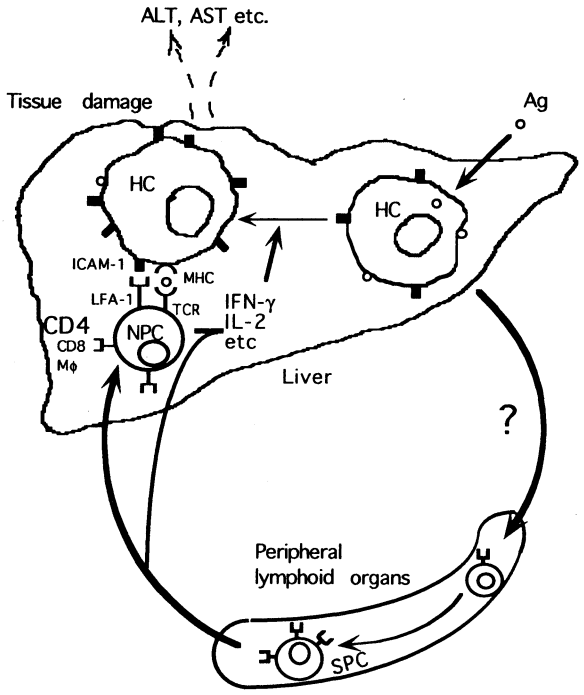

Fig. 1 Proposed process for hepatocyte damage in PCl-DTH liver injury mice. PCl challenge in the liver of PCl-sensitized mice may cause an increased expression of both LFA-1 and ICAM-1 molecules. LFA-1-positive lymphocytes infiltrate to the liver and enhance the expression of LFA-1 on NPC and ICAM-1 on HC. ICAM-1 expression on HC may be up-regulated by Th1-like cytokines such as IFN-γ and IL-2. Liver-infiltrating NPC induces HC damage through LFA-1/ICAM-1 interaction, leading to the ALT and AST elevation in plasma and liver tissue damage. Such effector cells are mainly $CD4^+$ and $CD8^+$ T cells.

I-4. Role of Th1 and Th2 cytokines in regulating the liver injury induced by delayed-type hypersensitivity to picryl chloride

The present study observed an interesting kinetics of several Th1 and Th2-like cytokines in the liver injury model induced by DTH reaction to picryl chloride. Serum IL-2 and IFN-γ appeared in the earlier stage with a peak production at 6 and 12 hr, while IL-5 and IL-4 reached their maximum levels at 18 and 24 hr of liver injury, respectively. By means of the co-culture assay between liver nonparenchymal cells (NPC) and hepatocytes (HC), both recombinant murine IFN-γ and IL-2 triggered the interaction between NPC or spleen cells and HC at 0 hr of liver injury and consequently led to a remarkable elevation of ALT in co-culture supernatant, with an enhancement in expressions of both LFA-1 on NPC and ICAM-1 on HC. Oppositely, the 10 hr-preincubation with IL-4 and IL-5 of NPC at 12 hr of liver injury completely abolished its hepatotoxicity without influencing the expressions of adhesion molecules. These results suggest that there is an involvement of Th1 and Th2 regulatory system in this liver injury, respectively contributing to the up- and down-regulation of hepatic damage. The acquisition of the hepatotoxic potential for liver-infiltrating cells by IL-2 and IFN-γ may be associated with the enhancement in the expressions of both LFA-1 and ICAM-1, and the roles of IL-4 and IL-5 might be reflected in the induction of the functional dysregulation of NPC. Such Th1 and Th2 balance may critically contribute to the production of or recovery from the liver injury.

II. PCl-DTH 肝障害を薬効評価モデルとしての有用性および肝炎新薬開発の new strategy

II-1. Liver injury model induced in mice by a cellular immunologic mechanism – Study for use in immunopharmacological evalua-

tions

Various drugs for clinical hepatitis were applied to a new model of liver injury induced in mice by the delayed-type hypersensitivity to picryl chloride (PCl-DTH). The hepatoprotective agent, biphenyl dimethyl dicarboxylate showed a remarkable improvement against the elevation of serum transaminase levels as well as the histopathological changes when given during the induction phase but not during the effector phase of DTH reaction. Cyclophosphamide (Cy), an immunosuppressive agent, significantly inhibited the enzymatic elevation given in both induction and effector phases. However, Cy did not affect the sustaining of liver injury 4 weeks after the liver injury eliciting. Moreover, the consecutive administration of prednisolone (Pred), in both induction phase and sustaining process of liver injury, conversely caused a more severe liver damage. Such exacerbation by Pred might be resulted from its toxic action to hepatocytes. As an immunomodulatory and antiinflammatory agent, glycyrrhizin remarkably improved the sustaining process but not the acute phase of the liver injury. Krestin and malotilate also showed an improving effect on the sustaining development of liver injury. These findings that most of above drugs showed an improving action in their respective manner suggest that this model may be useful for the pharmacological evaluation of drugs especially immunomodulating agents for hepatitis.

II-2. A new strategy for regulating the immunological liver injury—Effectiveness of DTH-inhibiting agents on DTH-induced liver injury to picryl chloride

Aqueous extracts from various crude drugs showing a selective inhibition on the induction or effector phase of delayed-type hypersensitivity (DTH) reaction were applied to the new model of liver injury induced in mice by picryl chloride (PCl)-induced DTH. The inhibiting drugs to the induction phase of DTH, *Fructus triburi* (FT) and Er-Miao-San (EMS), showed a remarkable improvement against the elevation in serum transaminase levels as well as in histopathological changes when given during this phase. The administration in effector phase by Rhizoma *Smilacis glabrae* (RSG) and Cortex *Dictamni* (CD), selectively inhibiting the phase of DTH, also significantly improved the liver damage. In addition, RSG and CD showed an almost complete recovery of serum alkaline phosphatase from a persistent decrease in the sustaining process of liver injury when given consecutively for 4 weeks after the elicitation of liver injury. Cyclophosphamide, an immunosuppressive agent, significantly inhibited the enzymatic elevation given in either of the phases, while did not affect the sustaining of liver injury. When above extracts were given in a combined manner to a same mouse during these two phases, respectively, FT with RSG and EMS with CD showed a distinct synergism against the liver injury. RSG or CD also enhanced the activity of prednisolone in suppressing PCl-induced ear contact sensitivity. These findings suggest that this immunological liver injury may be regulated by a set of selective suppressants to DTH reaction and the suitable application of such agents may pave a new strategy for treating liver damage.

III. 中薬土茯苓およびその成分 astilbin の薬理活性及び肝障害に対する新しい作用様式

III-1. A selective dysfunction of liver-infiltrating cells that cause hepatocyte damage by astilbin, a flavanoid isolated from Rhizoma Smilacis Glabrae, contributing to a new way of hepatoprotection from liver injury

The present study demonstrated that astilbin, a flavanoid isolated from Rhizoma *Smilacis Glabrae* (RSG), and aqueous extract from RSG (RSG ext) significantly improved the liver injury induced by DTH reaction to picryl chloride in mice, when administered during the effector but not induction phase. Their selective activity was associated with the dysfunction of liver nonparenchymal cells (NPC) that cause hepatocyte (HC) damage. Against the release in culture supernatant of transaminases from HC by NPC from liver injured mice, the pretreatment of NPC with the drugs caused a concentration- and time-dependent inhibition. Such dysfunction was also confirmed in astilbin or RSG ext-administered mice, where NPC isolated from the mice showed a significant incompetence of hepatotoxicity *in vitro,* as well correlat-

ed with the inhibition of serum ALT elevation. When the pretreatment was performed on HC, however, neither astilbin nor RSG ext blocked the hepatotoxicity. Similarly, 6-day preventive administration of them failed to protect CCl_4-induced liver damage in mice. Furthermore, astilbin or RSG ext significantly promoted the apoptosis of NPC at 12 hr of liver injury, whereas did not influence the NPC and spleen cells from naive mice. These results suggest that astilbin displays the improving activity against liver injury through a selective dysfunction of liver-infiltrating cells rather than through the hepatoprotective manner so far, and is one of the effective principles contained in RSG. Such characteristics of astilbin will be of significance to pave a new way for the therapy of immunologically-related liver diseases and for the development of new drugs.

Ⅲ-2. Anti-inflammatory activity of the aqueous extract from Rhizoma *Smilacis glabrae*

Our previous paper has reported that the aqueous extract from Rhizoma *Smilacis glabrae* (RSG) (RSG ext) selectively inhibited the effector phase of delayed-type hypersensitivity (DTH) without suppressing humoral immune response. In the present study, a remarkable inhibitory activity was exhibited by the extract against both primary and secondary hind paw swelling of adjuvant arthritis in rats. RSG ext also significantly reduced the inflammatory edema induced by carrageenan in either naive or bilaterally adrenalectomized rats, suggesting the independence of the anti-inflammatory action on the function of pituitary-adrenal axis. The PGE_2 content in the carrageenan-induced inflammatory tissue was also decreased remarkably by the extract. Furthermore, RSG ext showed a distinct inhibition on the formation of cotton-induced granuloma formation. However, as compared with a steroidal agent, prednisolone, the extract did not affect the vitamin C content in adrenal gland as well as the weights of some organs. These results suggest that RSG ext may act as a therapeutic agent of immunoinflammatory diseases through a selective suppression on the cellular immune response involved in inflammations as well as through a direct anti-inflammatory mechanism including inhibiting PGE_2.

◇ 原　著

1) Qiang Xu, Feihua Wu, Baoling Zhang, Jieyun Jiang, Jinfu Lu, Jingsong Cao, Rong Wang and Zhenqing Feng. One-shot delayed-type hypersensitivity reaction in the mouse liver causes a sustained liver injury to picryl chloride. *Life Sciences* 1997 ; 60 : 2417-2425.

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◇ 総　説

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◇ 学会報告

1) 徐強，蔣潔雲，呉斐華：遅延型アレルギーの抑制

から肝障害の制御へ　清熱利湿中薬を中心に．第14回和漢医薬学会大会招待講演，1997，8，大阪．
和漢医薬学雑誌 **1997；14(4)：253-256．**

【目的】 清熱，利湿などの効能を持つ中薬は主として皮膚，関節，器官などに発生する各種の炎症性疾患に使われてきている。演者らはいままで種々の中薬を用いて，遅延型アレルギー（DTH）反応に対する有効性を検討してきた。そのうち，DTHの各phaseを選択的に抑制するものをいくつか見い出し，そのメカニズムと有効成分の解明を試みた。一方，肝炎の発症と慢性化進行において$CD4^+$および$CD8^+$ T細胞を中心とする細胞性免疫学的機序の関与が知られている。我々はすでにこの細胞性免疫病理過程を模擬して新しい肝障害モデルを作成し，DTH反応と肝障害との関連を動物にて結びづけた。このモデルを用いて肝障害の発症と慢性化進展における細胞および分子機構を解明し，さらにDTH抑制作用を示す中薬を肝障害の改善または制御に活用する試みを行ったので報告する。

【方法と結果】 1．耳や足蹠で惹起するDTH反応に対して種々の中薬の作用を観察した結果，蒺藜はDTHのinduction phaseのみを抑制し，龍胆および二妙散は両phaseをも抑制したが，いずれも抗炎症作用は示さなかった。土茯苓，白鮮皮，苦参，地膚子，赤芍はeffector phaseを抑制したが，induction phaseにはほとんど影響を及ぼさなかった。

2．picryl chlorideによるDTH反応（PCl-DTH）をマウスの肝臓内に導入して細胞免疫性肝障害を惹起した。すなわち，マウスの腹部に1％PClのethanol溶液を5日間おきに2回塗布して感作し，その5日後に0.2％PClのolive oil溶液を肝内に注射して24時間後に血清生化学的および肝組織病理学的検査を行った。その結果，肝内におけるDTH反応の強度に依存して血清alanine transaminase（ALT）およびaspartate transaminase（AST）活性などが著しく上昇し，肝細胞壊死，炎症性細胞浸潤などの組織像も見られた。

3．PCl-DTH肝障害の発症機構として，脾細胞（SPC）及び肝内nonparenchymal cell（NPC）表面lymphocyte function associated antigen-1（LFA-1），ならびにparenchymal hepatocyte（HC）表面intercellular adhesion molecule-1（ICAM-1）の発現が有意に増加することを見い出した。発現のpeakはSPCでは肝障害惹起の6時間後，NPCおよびHCでは12時間後であり，血清ALT上昇のpeakは18時間後であった。肝障害惹起前の脾臓摘出によってHCにおけるICAM-1発現の抑制傾向がみられると同時に，血清ALT活性の上昇が有意に抑制された。HCをNPCまたはSPCとともにco-cultureすると，培養上清中にALTのreleaseが増加した。あらかじめanti-LFA-1でNPCまたはSPCを，anti-ICAM-1でHCをそれぞれ処置すると，NPCまたはSPCによるALT releaseの増加はほぼ完全にブロックされた。一方，anti-$CD4^+$抗体は補体処置を加えることによってこの増加を完全に抑制したが，補体無処置では抑制は示さなかった。

4．肝臓内に1回DTH反応を導入することにより惹起された肝組織障害は数ケ月も続いていることを見い出した。すなわち，血清lactic dehydrogenaseとprolidase活性および肝組織hydroxyproline含量は有意に増加し，血清alkaline phosphatase活性，albumin含量は著明に低下した。組織病理像では肝細胞壊死と炎症性浸潤が比較的長期間に存続したほか，肝細胞変性，結合組織増生などが現われ，一部のマウスではportal areaの拡大，さらにbridging necrosisまで至った。この慢性化進行に対して肝障害1週後のマウスSPCを同系マウスに肝障害惹起と同時に移入すると，recipientマウスの肝障害を有意に増強したが，3-8週後のdonorは逆に抑制を示した。これに対応してdonor脾細胞の$CD4^+/CD8^+$ ratioは1週後では増加したが，3週以後では著明に低下した。

5．上述のDTH抑制中薬を用いてPCl-DTH肝障害に対する影響を検討した結果，蒺藜，龍胆および二妙散はinduction phaseに，土茯苓および白鮮皮はeffector phaseに投与することによって，それぞれ肝障害を明らかに改善した。また，土茯苓および白鮮皮は長期投与によって肝障害の慢性化過程に対しても改善を示した。

6．土茯苓およびその成分astilbinでNPCを処置することによってNPCの肝細胞障害作用を濃度依存的に抑制した。一方，HCを処置してもNPCまたはCCl4による肝細胞障害作用に対して土茯苓およびastilbinのいずれもhepatoprotective活性を示さなかった。

【考察】 DTH反応を肝臓内に導入することによって肝障害が惹起された。この肝障害の発症に関して肝内に浸潤した免疫活性細胞，とくに$CD4^+$ T細胞がLFA-1/ICAM-1 interactionを介して肝細胞に障害を与えることを明らかにした。また，one-shotなDTH反応で肝障害の慢性化進行が観察され，$CD4^+$および$CD4^+$ T細胞がこの慢性化に対してそれぞれ正と負の調節を果たしていることを証明した。DTH抑制作用を有する種々の清熱，利湿中薬が特徴を持って肝障害を改

善し，DTHを抑制することから肝障害を制御する可能性が示唆された。これら中薬のうち，土茯苓およびその成分astilbinは従来の肝庇護薬と異なって，肝内に浸潤する免疫細胞に対して選択的に作用して肝障害改善効果を示すと考えられる。

2）徐強，蒋潔雲，呉斐華，藤猪英樹，済木育夫：遅延型免疫反応性肝障害の発症機構と中薬土茯苓の肝障害改善効果。第1回免疫薬理研究会，1997，9，岐阜.

【目的】我々はすでにマウスの遅延型免疫反応による新しい肝障害モデルを作成し，そのeffector細胞および肝障害の慢性化などについて報告してきた。本研究では，この肝障害の発症における細胞接着分子の役割について検討し，さらに中薬土茯苓の肝障害に対する改善効果を検討したので報告する。

【方法と結果】 1）BALB/cマウスの腹部に1％ picryl chloride（PCl）のethanol溶液 0.1 mlを5日おきに2回塗布して感作し，その5日後に0.2％ PClのolive oil溶液10 μlを肝臓内に注射して肝障害を惹起した。肝障害マウスの脾細胞（SPC）及び肝内nonparenchymal cell（NPC）のlymphocyte function associated antigen-1（LFA-1）の発現，ならびにparenchymal hepatocyte（HC）のintercellular adhesion molecule-1（ICAM-1）の発現は正常マウスの各細胞に比較して有意に増加していることを見い出した。その接着分子の発現peakは，SPCでは肝障害惹起の6時間後，NPCおよびHCでは12時間後であり，一方，血清 ALT上昇のpeakは18時間後であった。2）肝障害惹起の12時間前の脾臓摘出によって血清ALT活性の上昇は有意に抑制され，HCのICAM-1の発現も抑制傾向が見られた。3）HC をNPCまたはSPCと共培養することにより，培養上清中にHCからのALT遊離がみられた。このALT遊離作用は肝障害惹起6時間後のSPCおよび12 時間後のNPCを用いることにより最大となり，これらの細胞表面上のLFA-1の発現とよく一致した。4）共培養する前に，anti-mouse LFA-1抗体でNPCあるいは SPCを，anti-mouse ICAM-1抗体でHCをそれぞれ処置すると，NPCあるいはSPCによるALT遊離作用はほぼ完全に阻害された。一方，anti-$CD4^+$抗体は補体を加えることによりNPCのHCに対するcytotoxicityを完全に抑制したが，補体無処置では抑制されなかった。5）この肝障害モデルに対して中薬土茯苓の水性エキスは肝障害惹起後の経口投与によって有意な改善効果を示した。6）土茯苓の水性エキスおよびその成分astilbinでNPCを処置することによってNPCの肝細胞障害作用を用量依存的に抑制したが，HCを処置することによっては影響を及ぼさなかった。また，土茯苓水性エキスおよびastilbinはNPCおよびHCのviabilityに対していずれも影響を及ぼさなかった。

【考察】この肝障害モデルの発症において，肝内に浸潤した免疫細胞，主に$CD4^+$ killer T細胞がLFA-1/ICAM-1 interactionを介して肝細胞に障害を与えることを明かにした。また，土茯苓およびastilbinは肝細胞保護ではなく，肝内に浸潤する免疫細胞に選択的に作用して肝障害改善作用を示すと考えられる。