# 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細胞及び分子レベルで解明し，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。すでに肝炎の細胞性免疫病理過程を動物にて模擬し，遅延型アレルギー反応による新しい肝障害モデルの作成に成功した。また，この肝障害の慢性化持続および薬効評価モデルとしての有用性などを証明した。その上でこの肝障害における分子機構，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試みており，これらに対する和漢生薬の作用およびその活性物質についても研究を続けている。本年度の主な研究課題と成果は下記のとおりである。

**Ⅰ．肝障害の発症と進行に関する基礎的研究**

1）マウスにて遅延型アレルギー反応による新しい肝障害モデルを作成し，そのeffector cellの一つとしてThy 1.2$^+$，L3T4$^+$，Lyt-2$^-$ T細胞であることを証明した。

2）この肝障害の発症において，マウス脾りんぱ球（SPC）及び肝内nonparenchymal cell（NPC）表面lymphocyte function associated antigen-1（LFA-1），ならびにparenchymal hepatocyte（HC）表面のintercellular adhesion molecule-1（ICAM-1）の発現は有意に増加することを見い出した。

3）HCをNPCまたはSPCとco-cultureすることにより，培養上清中にalanine transaminase（ALT）のreleaseが認められ，この作用はanti-mouse LFA-1でNPCまたはSPCを，anti-mouse ICAM-1でHCを前処置することによって，ほぼ完全に抑制された。生体の免疫細胞がLFA-1/ICAM-1 interactionを介して肝細胞に障害を与えることを示唆した。

4）LFA-1 positive細胞のうち，とくにCD4$^+$ killer T細胞が肝細胞障害の惹起において重要な役割を果たすことを明かにした。

**Ⅱ．肝障害に対する中薬土茯苓およびその成分astilbinの影響**

1）土茯苓の水性エキスが遅延型免疫反応性肝障害を有意に改善した。

2）土茯苓の水性エキスおよびastilbinがNPCの肝細胞障害作用を有意に抑制した。

## Ⅰ．肝障害の発症と進行に関する基礎的研究

### I-1. 遅延型アレルギー反応による新しい肝障害モデルの作成およびその effector 細胞

Xu, Q., Wang R., Jiang J., Wu F., Lu J., Tan P.K., and Xu L.: Liver injury model in mice induced by a cellular immunologic mechanism − delayed - type hypersensitivity-induced liver injury to picryl chloride and phenotype of effector cell. *Cell. Immunol.*, 167 : 38-43, 1996.

Liver injury was induced in BALB/c mice by local delayed-type hypersensitivity (DTH) to picryl chloride (PCl). Distinct changes of biochemical parameters were observed including the elevation of serum alanine and aspartate aminotransferases, increase of liver lipid peroxides, as well as decrease of serum alkaline phosphatase. Damage was confirmed by histopathological findings such as hepatocellular necrosis, granulocyte infiltration, and fatty degeneration. The liver injury was passively transferred into naive syngeneic mice by infusing spleen cells from immune mice. The capacity of the splenocytes to induce liver injury in recipient mice was almost completely abolished by pretreatment

of the cells with anti-Thy 1.2 or anti-CD4, but not anti-CD8 antibody. These findings suggest that the production of liver injury by a local DTH mechanism is possible and the subpopulation of T cells, Thy $1.2^+$, $L3T4^+$, and $Lyt\text{-}2^-$ cells, is at least one of the effector cells that mediate the injury.

**Ⅰ－2．遅延型アレルギー性肝障害の発症における分子機構**

1) Increased expression of lymphocyte function-associated antigen-1 (LFA-1) on liver nonparenchymal cells (NPC) and spleen cells (SPC) and intercellular adhesion molecule-1 (ICAM-1) on parenchymal hepatocytes (HC) is observed in PCl-DTH liver injury mice.

The cell-cell interaction is the key process in the regulation of a variety of immune inflammations. To investigate the involvement of LFA-1/ICAM-1 pathway in the pathogenesis of PCl-DTH liver injury, we first determined the expressions of LFA-1 on NPC and SPC and ICAM-1 on HC by flow cytometric analysis. PCl-DTH liver injury was induced as follows. Briefly, mice were sensitized twice by painting 0.1 ml of 1 % PCl in ethanol on the skin of their abdomens at an interval of 5 days. Five days after the second sensitization, they were injected with 10 $\mu$l of 0.2 % PCl in olive oil into the liver to elicit the liver injury, followed 0 (liver injury non-elicited), 6, 12, 18 and 24 hr later by isolating the liver cells and spleen cells. The expression of LFA-1 antigen on SPC tended to be increased in the PCl-sensitized but liver injury non-elicited mice (0 hr) and rapidly reached a peak 6 hr after the PCl eliciting. The expressions of LFA-1 on NPC and ICAM-1 on 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 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.

2) PCl－DTH liver injury is reduced by splenectomy before eliciting.

Mice were done splenectomy 12 hr before the PCl eliciting in liver. A remarkable elevation of serum ALT activity was observed in the sham-operated control mice. To the control, a significant decrease in ALT was found in the splenectomy group. In this case, the ICAM-1 expression on HC tended to be decreased in the splenectomy mice against control while no influence was observed in LFA-1 expression on NPC.

3) Both NPC and SPC are in vitro cytotoxic against HC isolated from same mouse with PCl-DTH liver injury.

NPC and SPC were isolated from normal and PCl-DTH liver injury mice 12 hr after PCl eliciting. One hundred thousands of HC were pre-incubated for 5 hr and co-cultured with $4\times10^5$ and $1\times10^6$ of NPC or $5\times10^6$ of SPC for further 3 and 15 hr, respectively. As compared with the controls that were added with the corresponding amounts of NPC or SPC from normal mice, the co-culture with either NPC or SPC obtained from the liver injury mice caused a remarkable elevation of ALT and AST in the culture supernatant. There was in general no distinct difference in such elevation between 3 and 15 hr co-culture. The culture of NPC alone was also made and no detectable ALT activity was found in the supernatant. These results suggest that both SPC and NPC obtained from the liver injury mice show a direct cytotoxic activity to HC.

Additionally, neither NPC nor SPC from liver injury non-elicited mice caused the ALT release from HC. The ALT release from HC was shown by NPC obtained at 6 hr and reached a peak at 12 hr and declined at 18 and 24 hr after the eliciting. In the case of SPC, the release reached a peak 6 hr and was maintained in an almost same level 12 hr after. These changes in cytotoxic activity by NPC and SPC against HC seemed to be quite in accord with those in LFA-1 expression.

4) Binding to LFA-1 of anti-LFA-1, or to ICAM-1 molecule of anti-ICAM-1 before the co-culture abolishes the cytotoxic action of NPC and SPC to HC.

To assay the role of LFA-1/ICAM-1 interaction in the production of liver cell damage, NPC and SPC from PCl－DTH liver injury mice were pretreated with anti-LFA-1 antibody to bind the LFA-1 molecules on the cell surface. To the he-

patocytes from liver injury mice, $4 \times 10^5$ NPC caused an ALT release in a same extent with $5 \times 10^6$ SPC. Such ability of NPC and SPC to release ALT was completely inhibited by the pretreatment with anti-LFA-1. When the co-culture was conducted using the anti-ICAM-1 antibody-prebound HC, cytotoxicity was observed by neither NPC nor SPC. To normal hepatocytes, only a weak cytotoxicity was observed by NPC and SPC from liver injury mice and inhibited by the pretreatment with anti-LFA-1.

5) CD4 positive T cells cause the hepatocyte damage through LFA-1/ICAM-1 interaction.

NPC from liver injury mice was treated with various monoclonal antibodies before the co-culture with HC. The treatment by anti-Thy 1.2, -CD4 and -LFA-1 antibodies plus complement almost completely abolished the cytotoxicity of NPC to HC. The anti-CD8 and anti-Mac-1 antibody plus complement showed a tendency of inhibition. However, the treatment by these monoclonal antibodies without complement did not affect the cytotoxic action of NPC except for using anti-LFA-1 antibody.

Above results shown in 1)-5) suggest that the interaction between LFA-1 and ICAM-1 molecules is one of the important pathways in the pathogenesis of liver injury induced by PCl-DTH. The liver injury process may be supposed including the triggering by PCl challenge in the liver for increasing the expression of these molecules, the infiltration to liver of lymphoytes containing LFA-1 positive cells from the peripheral lymph organs, the cell-cell adhesion between the infiltrated cells and hepatocytes by LFA-1/ICAM-1 binding, and the cytotoxic action against parenchymal hepatocytes by the infiltrated cells mainly including $CD4^+$ killer T cells.

## II. 肝障害に対する中薬土茯苓およびその成分 astilbin の影響

The aqueous extract from *Rhizoma Smilacis Glabrae* (RSG), which has been evidenced to selectively inhibit the effector phase of PCl-induced ear dermatitis and other DTH reactions, showed a remarkable improvement of liver injury in this model when given po for 3 times 0, 5 and 10 hr after the PCl challenge in the liver. To investigate its mechanisms, the extract and its principle, astilbin have been used in the cell culture assay. As the result, neither the extract nor astilbin itself showed any cytotoxic activity to the isolated liver NPC or HC, but the pretreatment of NPC with either of them remarkably blocked the toxic action to HC. These results suggest that RSG and astilbin may improve the immunological liver injury through affecting the function of immune effector cells.

### 学会報告

1) Xu Q., Wu F., Lu J., and Jiang J.: Chronic development of delayed-type hypersensitivity induced liver injury to picryl chloride and effect of some drugs on it. The fourth Japan-China joint meeting on pharmacology, 1996, 10, p.48, Fukuoka.

The chronic development of liver injury induced by delayed-type hypersensitivity to picryl chloride (PCl-DTH) was observed in mice for over 9 weeks accompanying with the changes in various biochemical, immunological and histopathological parameters. Some of those were characteristic and significant such as the marked elevation of lactic dehydrogenase (LDH) activity in serum and hydroxyproline (Hyp) content in liver, and the dramatic decrease of alkaline phosphatase (AP) level in serum. Such changes could be exasperated by the systemic transfer of spleen cells from mice that were in the acute phase of liver injury but improved by that in the chronic phase of liver injury, paralleled with the phenotypic changes of T lymphocytes. At the same time, the hepatocyte regeneration, Kupffer's cell proliferation, connective tissue hyperplasia and bridging necrosis were observed remarkably. To the chronic development, glycyrrhizin, malotilate and PSK significantly recovered both declining of AP activity and increasing of LDH activity and Hyp content. The improvement of liver injury was also made by the Chinese herbal drugs, *Rhizoma Smilacis Glabrae* and *Cortex Dictamni,* Which showed a selective inhibition on the effector phase of DTH reactions. These results suggest that PCl-DTH liver injury could develop to a chronic state and T lymphocyte functions may be important for the development. Regulation on the cellular

immune functions such as DTH reaction may be one of the ways for developing a novel drug for hepatitis.

2) Qiang Xu : Therapy for hepatitis by traditional Chinese Medicine and new attempt for developing drugs. The 5 th International Symposium on Traditional Medicine. 1996, 11, p.3, 175-181. Toyama.

There remains lack of the special treatment and effective drugs for chronic hepatitis so far. In China, this kind of disease is usually treated by a combined therapy including Traditional Chinese Medicine (TCM). The therapeutic principles are generally selected in the case by TCM named as Qing-Re-Jie-Du, Huo-Xue-Hua-Yu, Fu-Zheng-Gu-Ben, etc. Many traditional Chinese medicines including the formulations that accord with the principles have been evidenced to be effective on the symptoms of hepatitis and some of them are also capable of promoting the negative conversion in HBV antigen-positive patients. It could be said, therefore, that TCM plays an important role in the treatment of clinical chronic hepatitis in China. On the other hand, an ideal animal model of liver injury is needed for elucidating the pathogenesis of hepatitis and for developing new drugs. For this reason, we have established a new liver injury model induced in mice by a typical cellular immune response, delayed type hypersensitivity to picryl chloride and clarified the effector cells involved in the model. Interestingly, the liver injury was found to be sustained for over 9 weeks and the biochemical, immunological and histopathological features in the chronic development were examined comprehensively. Furthermore, observations using various drugs for hepatitis also suggested that this model may be useful for the pharmacological study. By the model, some herbal drugs and their principles such as *Fructus Triburi, Radix Gentianae, Rhizoma Smilacis Glabrae, Cortex Dictamni* and Ruscogenoside C from the radix of *Liriope muscari* were proved to be effective on the liver injury. The cooperation effects were also observed between some of the drugs. Further investigations on the effective mechanisms of these herbal drugs as well as their effective components may help the development of more effective strategies and novel drugs for the treatment of the insidious disease.

**そ の 他**

1）徐　強：漢方理論の近代科学認識と動物モデルによる薬効評価．富山漢方会，1996，12，20，富山．