（第 3 号様式）

学 位 論 文 要 旨

氏　　名　姚　立穎

論 文 名

NAFLD モデルマウスにおける肝骨髄由来抑制細胞分画と免疫抑制機序の解析

学位論文要旨

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently one of the most commonly liver diseases worldwide in both adults and in children. Altered immunomodulation is thought to contribute to the pathogenesis of NAFLD.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells and comprise myeloid precursors. Recently, MDSCs have been found to accumulate in the liver of obese mice to suppress inflammation and maintain liver homeostasis; these MDSCs were identified as being $CD11b^{+}Gr1^{+}$ in mice. The Gr1 marker is a composite epitope between Ly6C and Ly6G antigens, and MDSCs can be further subdivided into $Ly6C^{+}$ monocytic and $Ly6G^{+}$ granulocytic MDSCs using these two antigens. However, other studies have reported that liver $CD11b^{+}Ly6C^{+}$ or $CD11b^{+}Gr1^{+}$ cells, categorized as macrophages, monocytes, or immature myeloid cells, contribute to liver inflammation, suggesting that the phenotype of liver MDSCs needs further investigation and specification.

The present study aimed to elucidate the profile of authentic monocytic MDSCs that accumulated in livers of NAFLD model mice and to assess their function with respect to T cell suppression and their role in the pathogenesis of liver inflammation in NAFLD.

Methods:

C57BL/6J mice were divided into control group which was fed a normal diet and NAFLD group which
was fed a high-fat diet. Liver non-parenchymal cell suspensions were analyzed using flow cytometry. $SSC^{high}CD11b^{+}Gr1^{dim}$ cells and $SSC^{low}CD11b^{+}Gr1^{dim}$ cells were sorted using FACS.

To investigate the suppressive function of MDSC, CFSE-labeled T cells were cultured with Dynabeads

氏名　姚　立穎

Mouse T-Activator CD3/CD28 in the absence or presence of sorted $SSC^{high}CD11b^{+}Gr1^{dim}$ cells or $SSC^{low}CD11b^{+}Gr1^{dim}$ cells. In some experiments, L-N6-(1-iminoethyl) lysine dihydrochloride, catalase, or N-hydroxy-nor-arginine, was added at the start of the cultures, respectively. Allogenic mixed lymphocyte reactions were used to confirm the suppressive ability of MDSCs. The nitric oxide (NO) concentration in co-culture supernatants was measured using a Griess reagents system.

CCL2 and M-SCF gene and protein expression were investigated using real-time RT-PCR and immunohistochamistry, respectively. MDSC migration ability was analyzed using CytoSelect™ 96-Well Cell Migration Assay. Bone marrow cells were cultured with or without recombinant M-CSF. After 3 days, $CD11b^{+}Gr1^{dim}Ly6C^{high}$ and $CD11b^{+}Gr1^{dim}Ly6C^{low}$ cells were sorted to allow testing of their function in the allogenic mixed lymphocyte reaction assay.

Results:

1) $CD11b^{+}Gr1^{dim}$ cells could be further divided into SSC^{high} and SSC^{low} populations. In NAFLD mice, the frequency of the $SSC^{low}CD11b^{+}Gr1^{dim}$ cells was significantly higher than that in control mice, and these cells were increased in the high fat diet mice over time.

2) $SSC^{low}CD11b^{+}Gr1^{dim}$ cells expressed $Ly6C^{high}$, CCR2, CD115, CD274, F4/80 and CD80. $SSC^{high}CD11b^{+}Gr1^{dim}$ cells expressed $Ly6C^{low}$, F4/80 and CD31. $CD11b^{+}Gr1^{high}$ cells expressed $Ly6C^{low}$ and Ly6G. $CD11b^{+}Gr1^{high}$ cells had lobular-shaped nuclei typical of granulocytes whereas $SSC^{high}CD11b^{+}Gr1^{dim}$ and $SSC^{low}CD11b^{+}Gr1^{dim}$ cells had ovoid nuclei typical of monocytes/macrophages.

3) $SSC^{low}CD11b^{+}Gr1^{dim}$ cells suppressed T cell proliferation; however, $SSC^{high}CD11b^{+}Gr1^{dim}$ cells did not affect T cell proliferation.

4) Addition of L-NIL, an iNOS inhibitor, restored T cell proliferation. The level of nitrite in supernatants of T cells or $SSC^{low}CD11b^{+}Gr1^{dim}$ cells alone was below the threshold of detection. However, after T cells and $SSC^{low}CD11b^{+}Gr1^{dim}$ cells were co-cultured, nitrite was produced in the supernatants at detectable levels.

5) CCL2 mRNA and protein expression level was increased in the livers of NAFLD mice. $SSC^{low}CD11b^{+}Gr1^{dim}$ cells migrated in response to CCL2 in a dose-dependent manner.

6) The mRNA and protein expression of M-CSF was increased in the livers of NAFLD mice compared with those of control mice. Bone marrow-derived $CD11b^{+}Gr1^{dim}Ly6C^{high}$ cells, which are phenotypically similar to SSC^{low} populations, were increased in the cultures that included M-CSF. These cells exhibited immunosuppressive ability.

Conclusion:

We identified $SSC^{low}CD11b^{+}Gr1^{dim}$ cells as the authentic phenotype of liver monocytic MDSCs and showed that these exhibit a strong suppressive ability on T cells. In addition, these cells inhibited T cell function through NO production by iNOS. Our results suggest that the accumulation of MDSCs in the liver might regulate the immune environment of NAFLD.

キーワード（３～５）	NAFLD, MDSCs, negative feedback