Acta Biomed 2011; 82: 146-153

Original article

Changes of spleen dendritic cells in the terminal stage of multiple organ dysfunction syndrome

Lu Jiangyang[1], Liu Qian[1], Wang Xiaohong[1], Yang Yi[1], Li Ling[1], Liu Yi[2],Yu Jia[2], Francesco Marotta[3], Chui Dehua[2]

[1] Department of Pathology, the 304 Division, General Hospital of PLA (Formerly 304 Hospital), Beijing, China; [2] Neuroscience Research Institute & Department of Neurobiology, Key Laboratory for Neuroscience, Ministry of Education and Ministry of Public Health, Peking University, Beijing, China; [3] ReGenera Research Group, Milan, Italy

Abstract. Immune dysfunction is associated with the onset of multiple organ dysfunction syndrome (MODS). To study the immune damage in the spleen, we observed pathological features of the spleen and investigated the number of splenic dendritic cells and T lymphocytes in MODS patients, 9 human MODS cases and 25 normal spleens were examined by light microscope, electron microscope and immunohistochemistry (S-100, CD11c, CD205, CD1a, CD80 and HLA-DR). There was resolution and dispersion of splenic corpuscles, especially white corpuscles, accompanied by apoptosis of a large amount of lymphocytes and increased number of splenic dendritic cells (DCs). $CD1a^+/S\text{-}100^+$ DCs and $CD205^+/S\text{-}100^+$ DCs increased but the $CD80^+/CD11c^+$ DCs and $CD1a^+/HLA\text{-}DR^+$DCs decreased in MODS patients($p<0.01$), $CD80^+/CD11c^+$ DCs and $CD1a^+/HLA\text{-}DR^+$DCs were mainly surrounding the remote periarterial lymphoid sheath and in red pulp. The ratio of $CD4^+/CD8^+$ T lymphocyte declined markedly. The results indicated that at the terminal stage of MODS, the spleen was seriously damaged, the splenic DCs were inactivated and many lymphocytes were lost, especially $CD4^+$T, which induced T cells incapacitation and immune suppression. It is suggested that there is an important relation between changes of splenic dendritic cells and loss of lymphocyts and pathogenesis of MODS. (www.actabiomedica.it)

Key words: multiple organ dysfunction syndrome (MODS); dendritic cell (DC); spleen

Introduction

Multiple organ dysfunction syndrome (MODS) means dysfunction even failure of more than two organs or systems induced by severe infection, on top of an injury or disease. It was a thorny problem and became a focus of investigation in critical care medicine due to its high morbidity and poor prognosis (1, 2). The pathogenesis of MODS is complicated. In recent years, serious disturbances in the immune system in the course of serious sepsis and MODS have attracted more attention of the investigators, immunosuppression and immunological dissonance were thought to play important roles in the pathogenesis of MODS (3-5). Previous studies showed that B lymphocytes and T lymphocytes as well as dendritic cells lost in lymphoid organs in sepsis patients (3, 6, 7), but the immunoreactivity of splenic dendritic cells in situ in MODS patients have not been reported. In this study, we collected 9 spleen specimens from 9MODS cases to study the characteristics of pathological changes of the spleen and examined the immunoreactivity of

This work was supported by grant 01MA210, NSFC30670414, NNSF Grant No. 30570533 and No. 30670414; 973- project No. 2006CB500705; 863- project No. 0060102A4031

splenic DCs in situ including the number changes and expression of co-stimulatery MHC-II,CD80 on spleenic DCs and change of lymphocytes.

Materials and methods

Specimen

20 control spleen specimens were obtained by splenectomy due to traumatic rupture of the spleen and another 5 from autopsy patients died of acute cardiac or cerebrovascular disease 1-3h after death. All these patients did not have severe wound infection or history of chronic disease.

The records of 9 patients died of MODS with total burn surface area (TBSA) as a result of serious injury, burn, infection and sepsis were collected and the diagnosis met the internationally consensual diagnosis criteria (8). MODS were diagnosed according to the criteria for MODS (1) (Table 1). Autopsy was done in 10 patients and spleen specimens were obtained by aspiration biopsy less than 30 minutes after death. Clinical immunologic tests before death showed hypofunction of cell-mediated immunity. All cases were performed with informed consent by the patients or his/her family member and follow the guidelines for experimental investigation with human subjects.

Light microscope

All spleen specimens were fixed in 10% formalin, paraffin-embedded and stained with Haematoxylin and Eosin after sectioning.

Transmission electron microscope

10 spleen specimens were observed with transmission electron microscope in the MODS group and they were processed for observation less than 2h after death. 12 cases in the control group ultrastructure ware examined. All these specimens were fixed with 3.1% glutaric dialdehyde, double-stained with lead and uranium. Model JEM-1200EX or JEM-1010 transmission electron microscope was used.

Immunohistochemical staining

The splenic DCs were marked by EnVision method with the double labeled technique for CD1a and S-100, CD205 and S-100, CD80 and CD11c, HLA-DR and CD1a. antibodies used were listed in Table 2. The double labeling Kit was purchased from Zhongshan biological reagent company. Staining was done according to the manuscript. All staining was done at room temperature in a humidified chamber. Dilutions and washings were done in PBS. After a 1-h incubation with the first primary Ab, the slides were incubated with Bio-Goat anti M/R for 40 min and streptavidin-AP for 30min, after each incubation, the slides were washed three times. NBT/BCIP was used to show the positive purple particles. And the then slides were incubated with the second primary Ab for 1-h, Bio-Goat anti M/R for 40 min and streptavidin-HRP for 30min, after each incubation, the slides were washed three times, AEC was used to show the positive red particles.

Table 1. Profiles of patients with MODS

No.	gender	Age (years)	No. of days MODS	Cause of MODS
1	F	20	13	82% TBSA*,50% full thickness burn area with bronchial pneumonia
2	M	37	7	60% TBSA,50% full thickness burn area with pyocyaneum bacterium sepsis
3	M	40	7	98% TBSA,92% full thickness burn area
4	M	41	5	90% TBSA,79% full thickness burn area
5	M	42	5	75% TBSA, 60% full thickness burn area
6	F	45	20	97% TBSA,92% full thickness burn area with septicopyemia
7	M	45	5	95% TBSA,79% full thickness burn area with respiratory tract burn
8	M	59	3	65% TBSA, severe respiratory tract burn and cerebral blast injury
9	M	68	11	70% TBSA, 53% full thickness burn area with Gram-Negative bacillus septicemia
10	M	41	8	91% TBSA,78% full thickness burn area

* TBSA, Total Burn Surface Area

Table 2. Antibodies used for immunohistochemistry

Antibody	Localization	Clone	Concentration for labeling	Source
CD1a	Cell membrane	O10	1:50	Dako
S-100	Cytoplasm	Rabbit	1:50	Dako
CD205	Cell membrane	DEC-205	1:80	Novocastra™
CD11c	Cell membrane	5D11	1:50	Novocastra™
HLA-DR	Cell membrane	TAL.1B5	1:50	Dako
CD80	Cell membrane	Rabbit	1:100	Boster
CD3	Cytoplasm	M7254	1:80	Dako
CD43	Cell membrane	DF-T1	1:80	Dako
CD20	Cytoplasm	L26	1:80	Dako
CD4	Cell membrane	4B12	1:20	Dako
CD8	Cell membrane	C8/144B	1:80	Dako

Markers for lymphocytes, CD3, CD43, CD20, CD4 and CD8 were purchased from Dako Company. Envision immunostaining was used according to method of Dako Company. Ten high power fields were selected at random at the spleen margin and all the cells and positive staining cells were counted. Ratio of positive cells= the number of positive cells/the number of all cells×100 (or 1000‰).

Statistical analysis

Data are reported as the mean ±SEM. Data were analysed using the statistical software SPSS13. Differences were considered significant at $p<0.05$

Results

Pathological changes in spleen of MODS patients

In the MODS group, splenic corpuscles were depleted obviously or even vanished in 10 cases (8/10, 80%). The area of white pulp diminished and was devoid of germinal centers, with sparse lymphocytes (Fig. 1B). The demarcation between the white pulp and red pulp became indistinguishable with blurred margins, most of periarterial lymphatic sheath had disappeared or formed sleeve-like hemorrhagic zone (Fig. 1C). In 10 cases (7/10, 70%), there was small focal or patchy hemorrhage and there were dissolution of splenic cords and phagocytes of erythrocytes. In 10 cases (9/10, 90%), the number of lymphocytes was obviously diminished in the white pulp and only a few lymphocytes were seen scattered around the arteries. There were many hyperplasic dendrite-like polygonal monocytes in the marginal zone of the white pulp or lymphoid tissues around the artery, and they were greater in number and larger in size compared with those in the control group with small lymphocytes around them (Fig. 1D,E). In some cases (4/10, 40%), there was heavy infiltration of neutrophils in the splenic sinus and splenic cord (Fig. 1F), and also hemorrhagic foci. By electron microscope, the surfaces of DCs had several protrudes. The nuclei were a little long and there were intentations on the surface of them. The electron- lucent cytoplasm was seen in the control spleen (Fig. 2, Left). The dendritic cells in the MODS spleen showed shrinkage, shortening of protrusions, pyknosis of cytoplasm (Fig. 2, Right).

Splenic dendritic cells increase in MODS patients

In the control group, there were $CD1a^+/S\text{-}100^+$ DCs in the white pulp and splenic cord, the number of DCs was the largest in the marginal zone (Fig. 3A, Right). Red fine grains were found dispersed in the membrane of CD1a positive cells and black grains in the cytoplasm of S-100 positive cells. In the MODS group, $CD1a^+/S\text{-}100^+$ DCs were more in size and larger in number compared with the control group, and they spread widely (Fig. 3A, Left, Table 2, $p<0.01$).

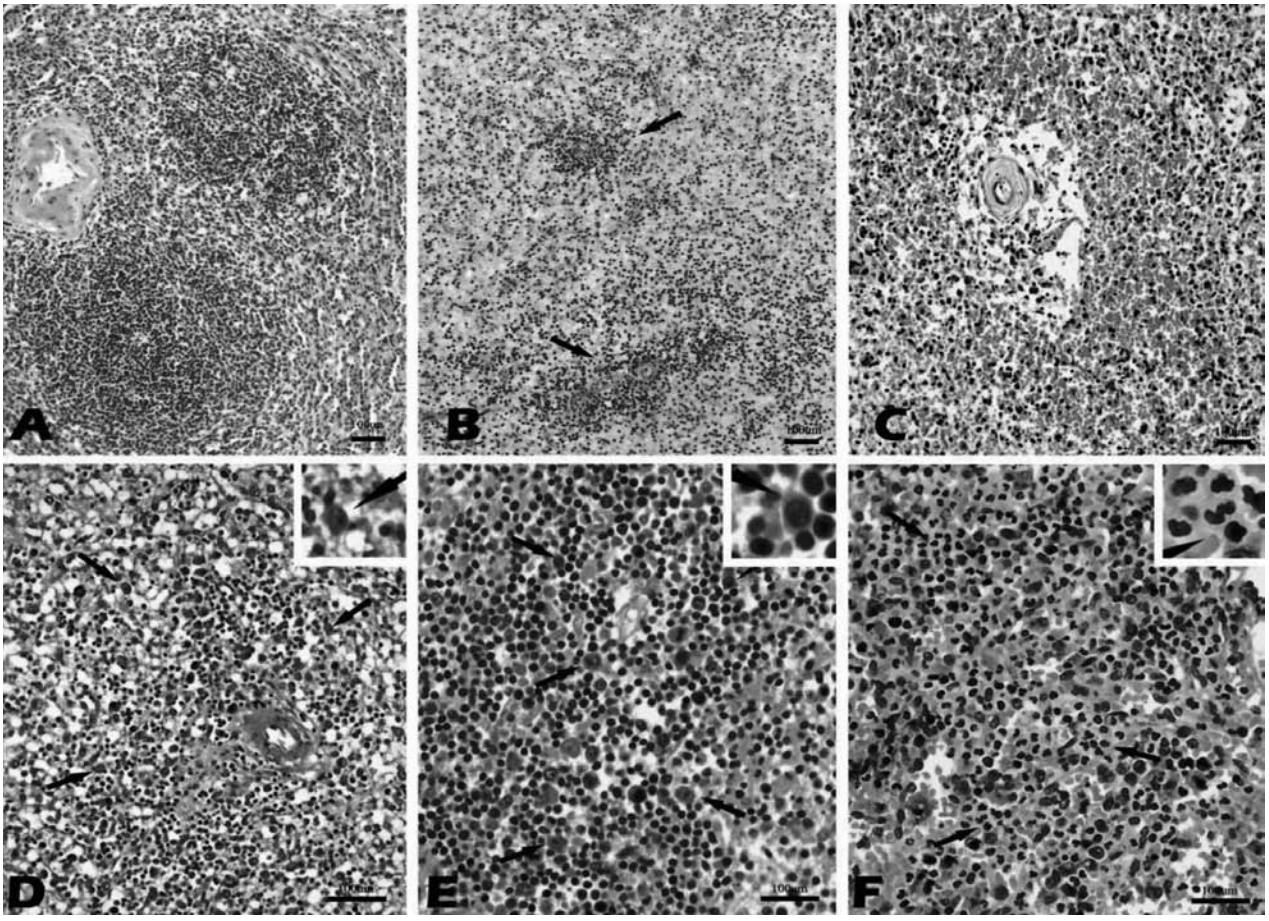

Figure 1. H&E staining in spleen tissues. (A) Control spleen tissue HE staining. (B) Serious multiple injuries complicated by MODS: the white pulp (↑) diminished, lymphocytes were sparse and splenic corpuscle vanished. (C) Abdominal injury with intraperitoneal abscess complicated by MODS: white pulp dispersed and there was sleeve-like hemorrhagic zone around the artery. (D) 95% TBSA, 79% degree burn complicated by MODS: there were less lymphocytes around the artery, among them there were hyperplastic dendrite-like polygonal monocytes (↑). (E) Serious infection complicated by MODS: in the marginal zone of the white pulp there were a lot of the hyperplastic dendrite-like polygonal monocytes (↑). (F) Serious lung infection with septic shock: there were neutrophils infiltration (↑) and hyperplastic dendrite-like polygonal monocytes in the splenic sinus and red pulp. (A-C, original magnification, ×100; D-F, original magnification, ×200; inserts, original magnification, ×400)

In the control group, there were $CD205^+/S\text{-}100^+$ DCs seen in the white pulp and the cords of red pulp, but most of DCs were located in the marginal zone (Fig 3B, Right). CD205 appeared as red fine grains spread in the cell membrane and also in the cytoplasm of positive cells, and S-100 spread in the cytoplasm of positive cells. In MODS group, $CD205^+/S\text{-}100^+$ DCs were more compared with the control group (Table 3, $p \leq 0.05$), and they spread mainly among the remaining lymphocytes around the periarterial lymphoid sheath and splenic cord in red pulp (Fig. 3B, Left).

Antigen presenting molecules of dendritic cells decrease in MODS patients

In the control group, there were many $CD80^+/CD11c^+$ DCs in the white pulp and the red pulp, and mainly in the marginal zone (Fig. 3C, Right). Red and purple fine grains spread in the cytoplasm and/or cell membrane of CD80 and CD11c positive cells. In the MODS group, the demarcation between the white pulp and the red pulp had become inconspicuous. There were few $CD80^+/CD11c^+$ DCs surround-

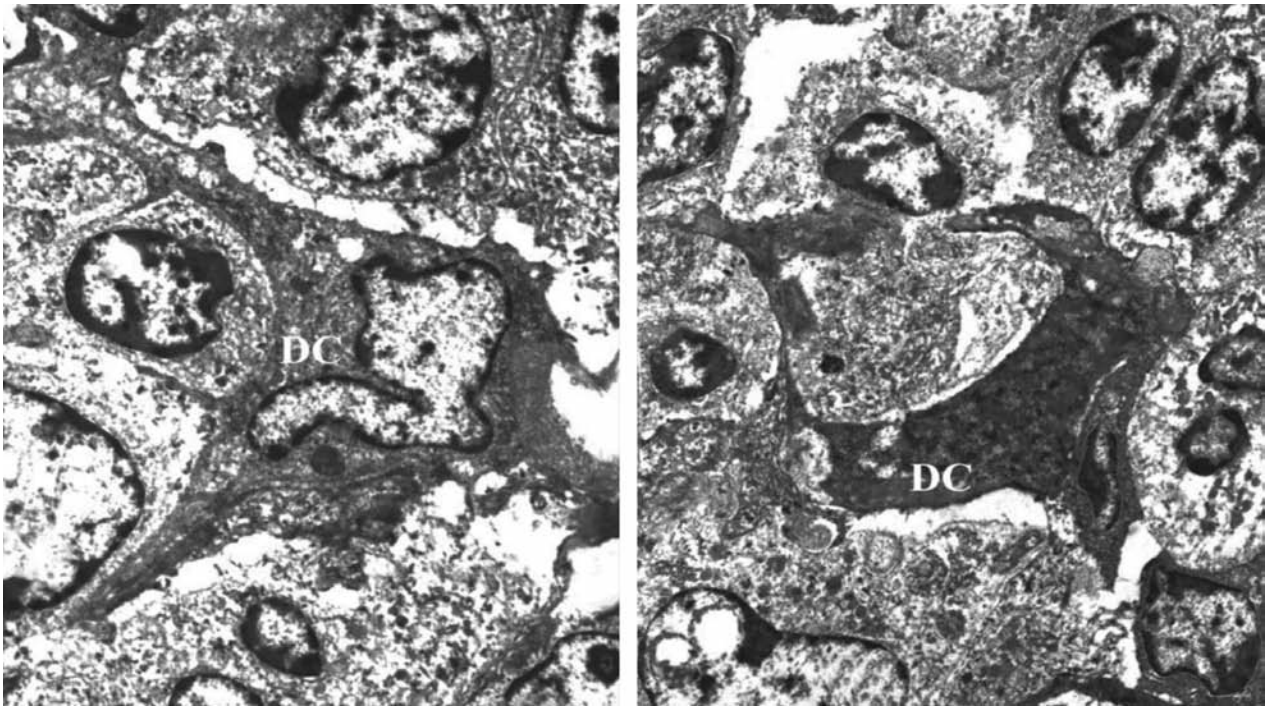

Figure 2. Ultra-structure of spleen dendritic Cells in normal control (Left) and MODS spleen (Right). (Left)In normal spleen, dendritic cells **(DC)** had protrusions. (Right)In a patient with 80% degree burn complicated by MODS: splenic dendritic cells **(DC)** showed atrophy and pyknosis of nuclei, increase in density of cytoplasmic electrons, condensation of organelles. (EM, Original magnification, ×5000. DC, dendritic cell)

ing the periarterial lymphoid sheath (Fig. 3C, Left), and there were much less $CD80^+/CD11c^+$ DCs compared with the control group (Table 3, $p<0.01$).

In the control group, there were large number of $CD1a^+/HLA\text{-}DR^+$ DCs in the white pulp and the red pulp, mainly located in the marginal zone and lymphatic follicles (Fig. 3D, Right). The cytoplasm and/or cell membrane of HLA-DR and CD1a positive cells were stained red and black. There were a few $CD1a^+/HLA\text{-}DR^+$DCs surrounding the remote periarterial lymphoid sheath and red pulp (Fig. 3D, Left) and the number of $CD1a^+/HLA\text{-}DR^+$DCs was remarkably diminished compared with the control group (Table 3, $p<0.01$).

The ratio of splenic CD4+ T lymphocytes decreases in MODS patients

In the MODS group, $CD3^+$T lymphocytes, $CD43^+$T lymphocytes and $CD20^+$B lymphocytes were less in number compared with the control group (Table 4, $p<0.05$); $CD4^+$T lymphocytes and $CD8^+$T lymphocytes, especially $CD4^+$T lymphocytes, decreased remarkably (Table 4, $p<0.01$), so the ratio of $CD4^+/CD8^+$T lymphocytes declined (ratio≤0.5).

Discussion

The pathogenesis of MODS is complicated (2, 3, 4). At present, it is accepted that the systemic inflammatory response resultant from trauma, major operation, or virus infection is so serious that it injures the host's organs, ending in MODS (5, 6). Recent studies showed that the imbalance of inflammatory and anti-inflammatory responses caused by over reaction of immune cells at the early stage of injury and infection is the major cause of sepsis; and the exertion of immune system and the inflammation out of control would damage the immune system. All of these would develop to immunosuppression or dissonance at the later stage, terminating in MODS (2-4, 7, 8).

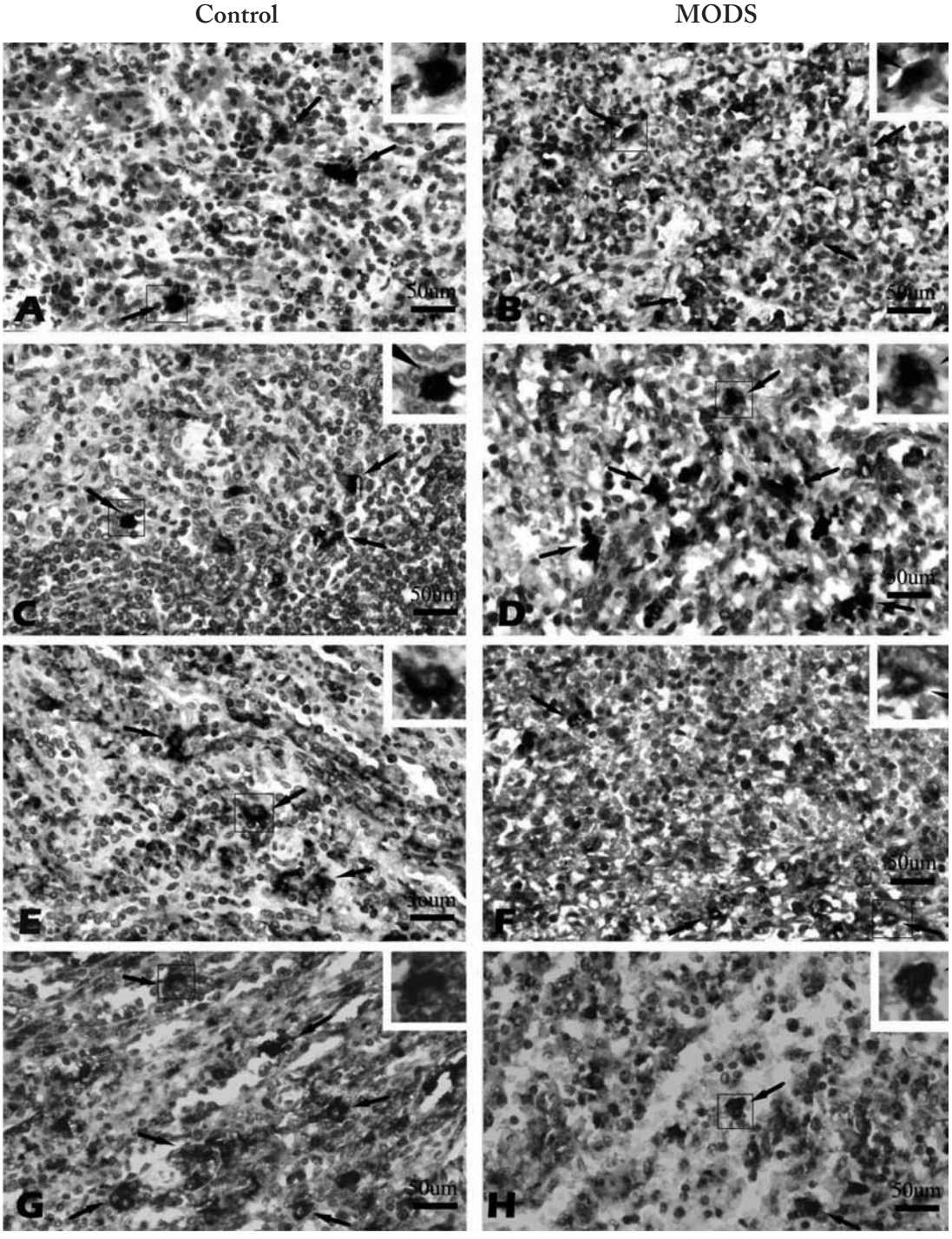

Figure 3. Immunohistochemical staining of normal control and MODS spleens. In normal control, a few CD1a+(red)/S-100+(purple) DCs (↑) (A) and few CD205+ (red)/S-100+ (purple) DCs (↑) (C)were observed in the margin area of the white pulp. Many CD80+(red)/ CD11c+ (purple) DCs (↑) (E)in the margin of the white pulp and many CD1a+(red)/ HLA-DR+ (purple) DCs (↑)(G) in the white pulp. In MODS spleens, hyperplastic and larger CD1a+/S-100+ DCs (↑)(B) and CD205+/S-100+ DCs (↑) (D)were observed in white pulp and red pulp; A few CD80+/CD11c+DCs (↑)(F) and CD1a+/HLA-DR+DCs (↑)(H) were in the dispersed white pulp. (Original magnification, ×200, inserts,×400)

Table 3. Percentage of splenic dendritic cells (Mean ± Sem) in control and MODS patients by immunohistochemstry assay

Antibodies	CD1a/S-100	CD205/S-100	CD80/CD11c	HLA-DR/CD1a
Control group	2.90±0.52	3.12±0.42	4.12±0.11	6.18±1.22
MODS group	5.98±0.32**	6.63±0.21*	0.98±0.17**	2.13±0.23**

*P<0.05 compared with control group; ** P<0.01 compared with control group

Table 4. The Percentage of different T lymphocyte subtypes in MODS spleens (Mean ± Sem)

	CD4+T lymphocyte	CD8+T lymphocyte
Control group	128.10±5.22	102.66±4.62
MODS group	21.02±1.15**	49.11±2.31*

* P<0.05 compared with control group; ** P<0.01 compared with control group

In this study, we collected 55 MODS cases caused by serious trauma, burn, major operation accompanied by bacteria, fungus or virus infection and studied retrospectively the changes of histological structure of spleen. It showed that the pathological changes in the spleen at the stage of MODS had common pathological features: 1) disintegration of periarterial lymphoid sheath and lymphocyte apoptosis. $CD3^+$ and $CD43^+$ T were less compared with the control group. $CD4^+$T and $CD8^+$T, especially $CD4^+$T lymphocytes, decreased remarkably; 2) atrophy or disintegration of lymph follicle (splenic corpuscle), $CD20^+$B decreased remarkably; 3) leukocyte infiltration and hemorrhage in the splenic sinus and red pulp in some cases; 4) DCs hyperplasia accompanied by degeneration and atrophy. These findings suggested that the spleen, which was mainly damaged by the general body reaction, was also the target organ of MODS; and it was the same mechanism inducing the body action that underlied the spleen damage, which were induced by different primary injuries.

In MODS, the lymphoid organs were seriously damaged, both the clinical and experiment showed dysfuntion of immune cells, especially T lymphocyte anergy. But the mechanism of the injury was unknown. In our early studies on the immunopathological changes in MODS animal, we discovered that pathological changes in spleen accompanied, and even affected the development of MODS (7-9). A few autopsy reports of MODS patients also showed there was serious damage to the peripheral immune organs such as spleen and lymph nodes induced by coronovirus infection (10). The functional status of spleen and other peripheral lymphoid organs is important for maintaining the balance of immune reaction and inflammatory response. As a major kind of antigen-presenting cell (APC), DCs reside in all lymphoid organs and are the initiating cells playing key roles in initiating immunoreaction (11, 12). We studied dynamically the effects of dendritic cells of the thymus and spleen in the progress of MODS. we found that the changes in the amount and activity of dendritic cells were important for establishing immune reaction and maintaining the body immune balance, and the status of dendritic cells affected directly or indirectly the function of immune organs and whole cell-mediated immunity of the body (7, 8). In this study we examined the expression of co-stimulatery molecules by double staining.

S-100, CD11c, CD1a and CD205 are known as the markers of human dendritic cells. CD205 is a type 1 cell-surface protein that belongs to a family of C-type multilectins and is expressed by a number of different types or subpopulations of dendritic cells (DCs). The expression of CD205 is up-regulated during DC maturation, which implies an important function for the molecule related to the maturation stage of the DC. In this study, $CD1a^+$/$S\text{-}100^+$ DC and $CD205^+$/$S\text{-}100^+$ DC in the spleen of MODS group were more than those in the control group.

Costimulatory molecule CD80 is expressed by mature DCs, and it reflects the ability of DCs to stimulate antigen-specific immune reaction. HLA-DR, one of the human major histocompatibility complex class II, reflects the functional status of antigen-presenting-cells (APCs), and HLA-DR is expressed at a high level by mature APCs on their surfaces. In this study, the number of $CD80^+$/$CD11c^+$ DCs and CD1a+/HLA-DR+ DCs declined significantly in spleen. It indicated that DCs in spleen were inactivat-

ed in MODS patients, hence impairs the cell mediated immunoreaction, inflammation and immunoreactivity.

In conclusion, the histological structures of the spleen as well as splenic dendritic cells are seriously damaged in MODS patients. The pathological changes in the spleen are finally about the same in MODS patients though the primary injury or diseases are different. It suggests that the different primary injury can cause the same body reaction which damages the spleen. Our foundings support systemic inflammatory response is the direct cause of spleen damage. Splenic DCs down-regulate HLA-DR and CD80, which prefer to induce immunoresistance and play important roles in the immune suppression in MODS patients. This study provides the pathological changes of spleen and splenic DCs in MODS patients and suggests that splenic DC may play important roles in the occurrence of immunosuppresionslose in MODS patients (13).

Acknowledgement

This work was supported by grant 01MA210 and NSFC30670414. This project was supported in part by National Natural Science Foundation of China (Grant No. 30570533; No. 3067414 and 985-2-044-121) and the National Hightech Research and Development program of China 973- project (No. 2006CB500705).

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Accepted: August 10th 2011
Correspondence: Dehua Chui,
Neuroscience Research Institute,
Department of Neurobiology,
Key Laboratory for Neuroscience
of the Ministry of Education,
Peking University Health Science Center,
38 Xueyuan Road, Beijing 100083, China
E-mail: dchui@bjmu.edu.cn.