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HISTOLOGICAL CHANGES OF MOUSE SPLEEN AND LYMPH NODE BY CYCLOPHOSPHAMIDE*

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=국문초록=

Cyclophosphamide에 依한 mouse의 脾臟과 淋巴節의 組織學的 變化*

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抗癌劑로 잘 알려진 cyclophosphamide (CY)를 成熟한 마우스에 體重 kg當 300mg을 腹腔內에 投與하여 CY가 體重, 脾臟重量 및 末稍淋巴組織에 미치는 影響을 實驗하였다.

CY는 마우스의 體重을 若干 減少시켰으나 體重은 곧 回復되었다. CY가 脾臟重量에 미치는 影響은 顯著하였는데 初期엔 脾臟重量은 減少되었으나 곧 正常으로 回復되고 그 後 正常보다 더 增加되었는데 그 增加는 CY投與 20日이 지나서야 正常으로 되돌아왔다. 脾臟 및 淋巴節의 組織學的 形態에 미치는 CY의 影響은 多樣하였다. 即, 胸腺依存細胞가 位置하는 脾臟의 periarterial lymphatic sheath와 淋巴節의 paracortex는 骨髓由來細胞가 位置하는 follicles보다 1日 乃至 2日 늦게 消失되었으며 脾臟의 肥大가 最高에 達하였을때 脾臟이나 淋巴節의 構造는 同一한 淋巴樣細胞로 構成된 interstitial tissue로 代置되어 있었다. 脾臟의 重量이 正常化되어감에 따라 脾臟이나 淋巴節의 構造가 正常化 되어갔다.

이와 같은 本 實驗의 所見은 CY는 骨髓由來細胞 뿐만 아니라 胸腺依存細胞에도 影響을 마친다고 思料된다.

著者들은 前報[23]에서 CY에 依한 遲延性過敏反應의 亢進은 suppressor T cell의 除去에 基因한 것이라고 示唆하였는데 本 實驗結果는 이를 뒷받침해준다고 思料되었다.

Introduction

It is well known that lymphocytes recirculate from the blood to the lymph through peripheral lymphoid tissues such as spleen and lymph nodes and from the lymph to the blood through thoracic duct continuously[1]. when lymphocytes enter the peripheral lymphoid tissues during recirculation, T and B cells are trapped in the different areas of spleen and lymph nodes. So periarterial lymphatic sheath of spleen and paracortex of lymph node are composed of almost thymus-derived lymphocytes (T cells)

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and follicles of spleen and lymph node are composed of almost bone marrow-derived lymphocytes (B cells)[2].

Cyclophosphosphamide (CY) as an alkylating agent is very cytotoxic for actively dividing cells. CY has been widely used as a potent anti-neoplastic drug against experimental tumors and human cancers[3], and used also as powerful immunosuppressive agent[4]. Numerous studies have been shown that CY is primarily a B cell suppressant[5~9]. Although CY-induced B cell suppression in chicken has beeen well characterized, reports of the effect of CY on the T cell system are conflicting. For instance, CY has been interpreted to have no effect[6,10], a transient inhibitory effect[9,11], or a long-lasting inhibitory effect on T cell functions[12]. In mammalian models, CY exerts a preferential effect on B cell function and the T cell system has been generally considered spared by CY[8,13]. Although under certain circumstances, T cells seem clearly affected[4,13~20]. Decrease in B cell functions and increase in T cell functions after CY administeration have been described by several workers[14,21,22]. We reported previously that CY administration to adult mice resulted in severe impairement of antibody formation to sheep red blood cells (SRBC) and significant enhancement of delayed-type hypersensitivity to SRBC as measured by footpad reaction and suggested that CY-induced potentiation of cell-mediated immunity might be due to the inactivation of CY-sensitive suppressor T cells[23].

This study was designed to examine the effect of CY treatment on the morphological changes of lymphoid tissues in mice. We will present the evidence suggesting that not only B, but also T cell systems are affected by the administration of a single dose of CY to adult mice.

MATERIALS AND METHODS

Animals

ICR inbred female mice maintained in our animal room, 2 to 3 months of age, were used.

Drug

Cyclophosphamide (Endoxan; Asta-Werke, Brackwede, Germany) was dissolved in sterile saline immediately before use and injected by the intraperitoneal (i.p.) route as a single dose in amount of 300mg/kg body weight at the same time to all groups of mice.

Body and spleen weights

Five mice were selected and examined every day until 12 days or every other day until 24 days after CY treatment for the study of body and spleen weights change. At the same time, the macroscopic changes of the spleen were observed.

Histology

Spleen fragments and lymph nodes were fixed in 10% formalin solution, and paraffin sections were stained with hematoxylin and eosin.

RESULTS

Body weight changes

Body weight of female mice given CY 300 mg/kg body weight decreased 5 to 10% of the normal value, and returned to normal range by day 6 (Fig. 1).

Spleen weight changes

Spleen weights, however, dropped to below 10% of the normal value within 48 hour. Transient recovery was evident by 10 days, with an overshoot to nearly double the normal weight at 12 days and a gradual return to the normal range by 20 days (Fig. 1).

Histology

Periarterial lymphatic sheath of spleen, paracortex of lymph node, and follicles of spleen and lymph node were carefully observed. Two

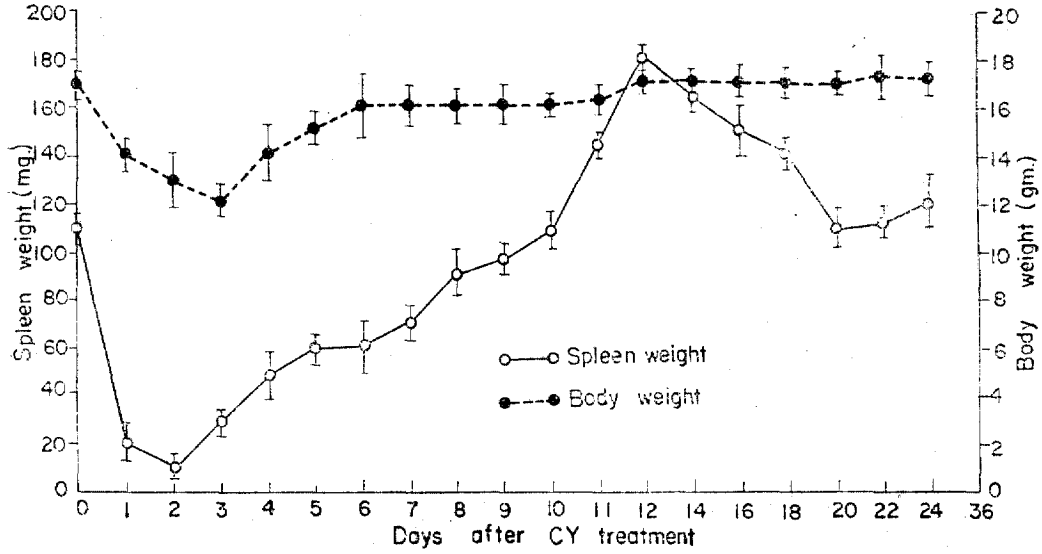

Fig. 1. Effects of CY on the spleen and body weights of ICR mouse. Each point represents mean of five mice and vertical bars indicate s. d.

days after CY injection, spleen became of small size with a dark red color, and all of the microscopic structures of both spleen and lymph node were not changed.

Five days of CY injection, the spleen became very small with a smooth surface. Follicles of spleen and lymph node disappeared, but periarteriolar lymphatic sheath and paracortex were recognized.

Seven days after CY injection, the spleen was still small and its surface became bristling with tiny nodules. Periarteriolar lymphatic sheath and paracortex began to be destroyed (Fig. 2 and 3).

There appeared some clusters of cells both in the subcapsular region and in the bulk of the

Fig. 2. Histology of the spleen of a ICR mouse treated with CY 7 days after injection (×100). Note the total destruction of follicles and the partial destruction of the periarteriolar lymphatic sheath.

Fig. 3. Histology of the lymph node of a ICR mouse treated with CY 7 days after injection(×100). Note the total destruction of the follicle and the partial destruction of the paracortex.

Fig. 4. Histology of the spleen of a ICR mouse treated with CY 9 days after injection (×100). Note the invasion of the spleen with a homogenous layer of lymphoid cells.

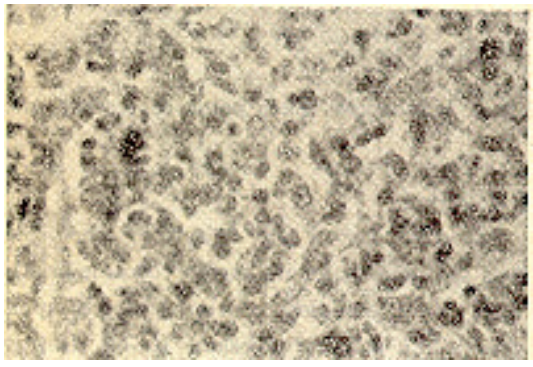

Fig. 5. Detail of Fig. 4(×400). Note the area of invading lymphoid cells with many large cells.

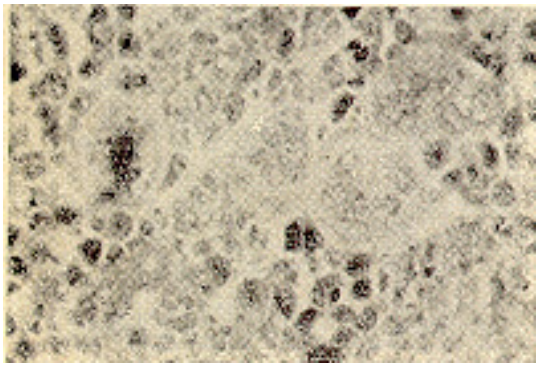

Fig. 6. Histology of the spleen of a ICR mouse treated with CY 11 days after injection (×400). Invasion of the spleen with homogenous layer of lymphoblasts and megakaryocytes.

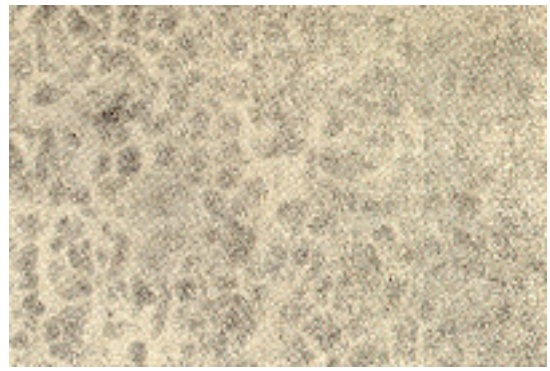

Fig. 7. Histology of the spleen of a ICR mouse treated with CY 20 days after injection (× 400). Note the homogenous layer of lymphoid cells with megakaryocytes.

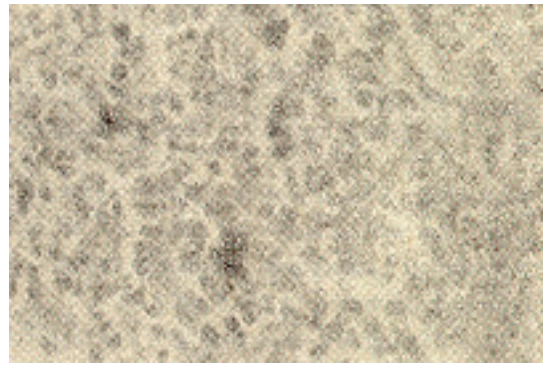

Fig. 8. Histology of the lymph node of a ICR mous treated with CY 20 days after injection (×400). Note the homogenous layer of lymphoid cells.

interstitial tissue of spleen. These cellsa ppeared to lymphoblasts with a clear nucleus, an abundent cytoplasm, and numerous mitosis (Fig. 4 and 5).

Eleven days after CY injection, the spleen was greatly enlarged, clear colored with a smooth surface, and easily dissociable. Periarteriolar lymphatic sheath and paracortex were almost destroyed, and follicles were absent. The interstitial tissue was composed of a dense and uniform layer of cells with a large nucleus and thin cytoplasm. The majority of cells were lymphoblasts with numerous megakaryocytes.

(Fig. 6).

Twenty days after CY injection, follicles began to reappear, but the spleen and lymph node were still constituted of a homogenous layer of lymphoid cells with many megakaryocytes (Fig. 7and 8).

Twenty-four days after CY injection, the architecture of spleen and lymph nodes were similar to normal with follicles, periarteriolar lymphatic sheath, and paracortex, but still magakaryocytes.

DISCUSSION

Cyclophosphamide (CY), a compound yielding a number of alkylating metabolites after activation by a microsomal enzyme system in the liver[24], has considerable antineoplastic activity against a variety of experimental tumors and human cancers[8], and is one of the most potent immunosuppressive agents[4]. The induction of a leukocytopenia, especially lymphocytopenia, with CY has been widely used in the human therapeutics. This differential effect of CY among circulating leukocytes depends on the different physiological states of the majority of the cells that make up the circulating leukocyte population. Only the lymphocyte among the peripheral leukocytes is not finally differentiated cell because CY has been shown to be most effective on the cell type that is under DNA synthesis[23].

There is considerable evidence in support of a selective action of CY on the humoral response (B cell dependent). The evidence for selectivity of CY on B cell was first reported by Lerman and Weidanz[6]. They showed that CY depressed selectively the development of the humoral response in newborn chickens as shown by the absence of significant IgM and IgG production with apparently normal cell-mediated immune response as measured in the graft-versus-host reaction. Their results were in agreement with the histologic observation reported by Stockman et al[4], which demonstrated that CY had caused selective depletion of B lymphocytes from lymph follicles, and germinal centers in lymph nodes and from equivalent thymus-independent areas in the spleen in the mouse. Also, Stockman et al[4], reported that the paracortex, thymus-dependent areas retained lymphocytes.

When Lagrange et al[14] reported that CY potentiate rather than inhibit the T cell dependent delayed-type hypersensitivity to sheep red blood cell (SRBC), there appeared some controversies over the mechanism of the potentiation of cell-mediated immune response by CY. Campa et al[25], Grozynski et al[26], and Bona et al[27] suggested that CY-induced potentiation of cell-mediated immune response was mediated by the relief of T cell function from B cell suppression. Alternatively, Lagrange et al[14], and Mackanese et al[28] reported that the enhanced delayed-type hypersensitivity in the CY treated mice was due to the relief of the T cell response from feedback inhbition that normally accompanies the development of the humoral immune response. Recently, however, the evidences are accumulating that the augmenting effect of CY on cell-mediated immune response is due to the inactivation of CY-sensitive suppressor T cells[22, 29~31] as the interaction of T-T cells in the immune response is considered an important key in resolving the regulatory mechanisms of cell-mediated immune response.

We previously presented some evidences that CY potentiated the cell-mediated immune response by the inactivation of CY-sensitive suppressor T cells [33]. Our present findings of histological studies showed that periarteriolar lymphatic sheath and paracortex(T-dependent areas) disappeared more slowly than follicles (B-dependent areas). Both T and B-dependent areas were destroyed finally by CY. However, all the functions of T cell subpopulations might not be affected the same with the findings of

a decreased graft-varsus-host reactivity in the residual T cell population reported by several workers [33-34]. In views of their reports, we suggest that CY may augment the cell-mediated immune response by elimination only the suppressor T cell activity. Our results of present study showed that lymphoid cells were found in the interstitial tissue, forming a homogenous layer, without follicular formation during spleen regeneration. It took over 24 days for the spleen to recover its classical structure with well developed follicles, and characteristic periarteriolar lymphatic sheath and paracortex. Our results clearly indicated that CY affected both B and T cell system rather than B cell system alone. We feel, however, further studies are deeded to clarify the nature and origin of the cellsrwhich appear during the step of regeneration of the spleen.

SUMMARY

Adult mice were injected with a single sublethal dose of cyclophosphamide. Effects of the drug on the body weight, spleen weight, and morphology of the peripheral lymphoid system have been analysed.

The body weights of the mice given cyclophosphamide (300mg/kg body weight) decreased slightly and returned to normal quickly. Spleen weights, however, changed greatly by keeping the process of decrease, recovery, overshoot, and gradual return to normal only by 20 days. Histologic examinations of spleen and popliteal lymph node showed that follicles disappeared 1 to 2 days before periarteriolar lymphatic sheath or paracortex. At the peak of splenomegaly, the architectures of spleen and lymph node were replaced with the interstitial tissue composed of dense and uniform layer of lymphoid cells. With the return of spleen weight to normal range, the architecturles returned to normal. Our results clearly indicated that cyclophosphamide affected not only B cells but also T cells.

These results seemed to suggest that augmentation of delayed-type hypersensitivity by cyclophosphamide may be due to the eliminateion of the suppressor T cells.

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