Therapeutic Effect of Cytomedicine on Mesangio-Proliferative Glomerulonephritis in Human Interleukin-6 Transgenic Mice

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Received October 7, 1996; accepted November 14, 1996

We previously demonstrated that IgG1 plasmacytosis in human interleukin-6 transgenic mice (hIL-6 Tgm) was suppressed by the implantation of SK2 hybridoma cells (SK2 cells, which secrete anti-hIL-6 monoclonal antibodies) microencapsulated in a semipermeable and biocompatible device. In this study, we demonstrated that the mesangio-proliferative glomerulonephritis in hIL-6 Tgm was also improved by the same treatment. These results strongly support the concept of cytomedicine, which is a novel drug delivery system (DDS) using living cells. However, an electron microscopy study showed that cytomedicine has a limited duration of effectiveness because of the disappearance of space for cell proliferation in the microcapsule. Thus, the control of cell proliferation in a device must be developed to prolong the function and effectiveness of cytomedicine.

Key words microencapsulation; drug delivery system; cytomedicine; human interleukin-6 transgenic mouse; SK2 hybridoma cell

With the remarkable advances of molecular biology in recent years, it has been gradually revealed on a molecular level that the living body is an aggregate of numerous cells which are highly differentiated in structure and function, with very complicated, specialized, yet interdependent functions to maintain homeostasis. It is especially surprising that bioactive molecules such as hormones and cytokines have temporal, quantitative, and local targets in a living body. These vital phenomena involve cells which produce and secrete bioactive molecules under various controls, connections, and networks. Regarding the bioactive molecules as drugs, the biosynthesis function, sensor function, and controlled release functions of bioactive molecules which are inherent in living cells are natural aspects of drug delivery systems (DDS). Therefore, it is not unreasonable to say that no medicine is superior to living cells in light of their functions and effectiveness. Because pharmacotherapy is presently performed with disregard to the homeostasis maintained by cell networks, patients suffer unfavorable side effects. Cell therapy, novel medical treatments against diseases using living cells, makes the best use of cell functions which naturally act as a DDS *in vivo*, and we propose an all-encompassing term for these cell DDS: cytomedicine.

We previously demonstrated that IgG1 plasmacytosis in human interleukin-6 transgenic mice (hIL-6 Tgm) was suppressed by a single injection of SK2 hybridoma cells (SK2 cells) microencapsulated in semipermeable and biocompatible materials, such as agarose hydrogel or alginate-poly(L)lysine-alginate (APA) membrane.^{1,2)} The hIL-6 Tgm of C57BL/6 origin, in which hIL-6 cDNA was introduced under a H-2L^d promoter, are subject to massive IgG1 plasmacytosis and mesangio-proliferative glomerulonephritis with age resulting from excessive activation of B lymphocytes and mesangial cells.^{1–5)} SK2 cells originally derived from BALB/c mice produce and secrete anti-hIL-6 monoclonal antibodies (SK2 mAb) which neutralize the hIL-6 biological activity.⁶⁾ Although this

cytomedicine was very effective as an *in vivo* long-term delivery system for bioactive molecules in this animal model experiment, it was also found that the effectiveness of this cytomedicine could not be permanently maintained.²⁾

In the present study, we examined the therapeutic effects of SK2 cells encapsulated in APA membranes (APA-SK2 cells) or agarose hydrogel (Aga-SK2 cells) on mesangio-proliferative glomerulonephritis in hIL-6 Tgm. APA-SK2 cells were observed by scanning electron microscopy on day 100 post-implantation to elucidate the reason the effectiveness of this cytomedicine had a limited duration.

MATERIALS AND METHODS

Animals C57BL/6 mice (female, 5 weeks old) and BALB/c mice (female, 5 weeks old) were purchased from the Shimizu Experimental Animal Co., Kyoto. The hIL-6 Tgm were kindly provided by Dr. Kishimoto (Osaka University Medical School, Osaka) and produced as described previously.^{3–5)}

Cells SK2 cells which produce and secrete SK2 mAb⁶⁾ were maintained by intraperitoneal serial passage in BALB/c mice which were intraperitoneally injected with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Wako Pure Chemical Co., Osaka) 1 week before inoculation of the cells.

Antibodies SK2 mAb were purified from ascites of BALB/c mice injected with SK2 cells with a Protein G Sepharose® 4 Fast Flow column (Pharmacia Biotech, Tokyo).

Microencapsulation of SK2 Cells within the APA Membrane or the Agarose Hydrogel SK2 cells were microencapsulated in APA microcapsules or agarose microbeads as described previously.^{1,2)}

Therapeutic Treatment of hIL-6 Tgm APA-SK2 cells (1.5×10^7 cells/mouse), Aga-SK2 cells (3.75×10^7 cells/mouse), or an unmicroencapsulated SK2 cell suspension

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Fig. 1. Therapeutic Effects of APA-SK2 Cells, Aga-SK2 Cells, Free SK2 Cells, and SK2 mAb on the BUN Level in hIL-6 Tgm

Three ml of APA-SK2 cells (5×10^6 cells per ml of capsules) or Aga-SK2 cells (1.25×10^7 cells per ml of beads) were intraperitoneally injected into hIL-6 Tgm at 7 weeks of age. Five hundred μ l of free SK2 cells (7.5×10^7 cells per ml) were intraperitoneally injected into hIL-6 Tgm at 7 weeks of age. SK2 mAb was subcutaneously injected into hIL-6 Tgm from 7 to 13 weeks of age at the dose of 50 μ g/mouse, 3 times/week. The BUN level was measured by Urea Nitrogen Test-Wako.

Fig. 2. Therapeutic Effects of APA-SK2 Cells, Aga-SK2 Cells, Free SK2 Cells, and SK2 mAb on Proteinuria in hIL-6 Tgm

The treatment of each group was as described in Fig. 1. The protein concentration in urine was measured by a Bio-Rad protein assay kit.

Fig. 3. Histological Appearance of Kidney in hIL-6 Tgm at 14 Weeks of Age with Periodic Acid Schiff Staining

A: Untreated hIL-6 Tgm (control group); B: free SK2 cells-injected hIL-6 Tgm; C: APA-SK2 cells-injected hIL-6 Tgm; D: Aga-SK2 cells-injected hIL-6 Tgm.

(free SK2 cells; 3.75×10^7 cells/mouse) in saline were intraperitoneally injected into hIL-6 Tgm (7 weeks old). For comparison of their therapeutic effect on mesangio-proliferative glomerulonephritis, 50 μ g of SK2 mAb was injected s.c. in hIL-6 Tgm (3 times/week between 7 and 13 weeks of age). Untreated hIL-6 Tgm or littermates were used as the control group and normal group, respectively. Each of these six groups was comprised of at least 4 mice.

Measurement of Blood Urea Nitrogen (BUN) Concentration and Protein Concentration in Urine The BUN level in plasma was monitored *via* tail-vein blood samples with the Urea Nitrogen Test-Wako (Wako). The protein level of the urine was monitored with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA).

RESULTS AND DISCUSSION

There was evidence that the untreated hIL-6 Tgm (control group) had severe nephritis after 14 weeks of age, since three mice of this group showed abnormal BUN values and the one remaining mouse had already died. In contrast, in the hIL-6 Tgm injected with Aga-SK2 cells or APA-SK2 cells, the BUN levels were suppressed to the normal level until 17 weeks of age. These therapeutic effects were equivalent to the result in the group of mice treated with SK2 mAb (50 μ g/mouse, 3 times/week, from 7 to 13 weeks old) (Fig. 1). The control group mice showed an increase in protein level beginning at 11 weeks of age,

and the protein content of this group at 15 weeks of age reached a level about 5-fold greater than that of their normal littermates. In contrast, in the hIL-6 Tgm injected with Aga-SK2 cells, APA-SK2 cells, or SK2 mAb, the protein level was suppressed to less than 1 mg/ml at 15 weeks of age (Fig. 2). Histological findings at 14 weeks of age revealed severe mesangio-proliferative glomerulonephritis with hyperplasia of the mesangial matrix and a proliferation of mesangial cells in the kidneys of the untreated control group and of the free SK2 cells-treated group (Fig. 3A and B). The renal structure, especially the structure of the tubule, remained almost normal in the APA-SK2 cells- (Fig. 3C) and Aga-SK2 cells- (Fig. 3D) injected hIL-6 Tgm. These data show that mesangio-proliferative glomerulonephritis was completely suppressed by the SK2 mAb secreted from intraperitoneal Aga-SK2 cells or APA-SK2 cells, which prevented hIL-6 from activating mesangial cells in the kidney. In a previous paper, we reported that Aga-SK2 cells- and APA-SK2 cells-injected hIL-6 Tgm demonstrated remarkable improvement of IgG1 plasmacytosis and a prolongation of survival time.^{1,2)} Moreover, APA-SK2 cells remained vital and secreted SK2 mAb in allogeneic recipients at 1 month post-implantation.²⁾ Thus, the cytomedical therapeutic effect on mesangio-proliferative glomerulonephritis in the present work is supported by these previous findings.

However, although these results demonstrated that the *in vivo* long-term delivery of bioactive molecules was

Fig. 4. Micrograph of SK2 Cells Entrapped in APA Microcapsules

APA-SK2 cells, 1×10^6 cells per ml of capsules, were intraperitoneally injected in C57BL/6 mice. On day 100 post-injection, APA-SK2 cells were retrieved from these mice and the surface (A, B) or the cross-section (C, D) of inner SK2 cells was photographed with scanning electron microscopy.

achieved by cytomedical therapy, the effectiveness of cytomedicine proved to have a limited duration. The cell viability of APA-SK2 cells implanted in allogeneic C57BL/6 mice gradually decreased after 1 month post-implantation, and could not be detected after 2 months post-implantation.²⁾ Therefore, in the present study we examined whether the APA membrane could immunoisolate inner SK2 cells from the host's immune system. Neither the cellular nor the humoral immune systems of allogeneic C57BL/6 mice were activated by the intraperitoneal injection of APA-SK2 cells, and the APA membrane completely protected the inner SK2 cells from the attack of immunocompetent cells in SK2 cell-immunized C57BL/6 mice (unpublished data). Thus, we presumed that this limitation of cytomedicine might be due to the disappearance of the inner space of the microcapsule for cell proliferation. When APA-SK2 cells recovered from C57BL/6 mice on day 100 post-injection were observed by scanning electron microscopy, the inner SK2 cells showed calcification due to overgrowth in the microcapsule, and the shape of the cells could not be distinguished on the outer surface of the cell cluster (Fig. 4A and B). Although healthy SK2 cells could also not be found inside the cell cluster, a few SK2 cells with a remaining cell shape were recognized near the center of the cell cluster (Fig. 4C and D). On the other hand, the surface of the recovered APA microcapsules was intact and no host's inflammatory response against APA-SK2 cells was observed (data not shown). An electron microscopy study of the encapsulated SK2 cells revealed that the control of cell proliferation in a device is necessary to cytomedicine in order to maintain function and effectiveness; for example, by utilizing cell lines which are sensitive to contact inhibition, the formation of spheroids, or the induction to terminal differentiation.

With the development of genetic engineering and cell technology, living cells can be induced to perform various functions.⁷⁻¹³⁾ Although further research is necessary, we consider that cytomedicine will revolutionize drug therapy once cytomedicine using engineered cells micro-

encapsulated in a biocompatible, semipermeable membrane is developed for clinical use as the next generation of DDS.

Acknowledgments We are grateful to Dr. T. Kishimoto at the Osaka University Medical School for supplying us with hIL-6 Tgm. We also thank Ms. Yuriko Kimura for her technical assistance on an electron microscopy study.

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