Prolongation of the Effective Duration of Cytochemical Therapy by Re-injecting SK2 Hybridoma Cells Microencapsulated within Alginate–Poly(L)lysine–Alginate Membranes into Human Interleukin-6 Transgenic Mice

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We previously reported that SK2 hybridoma cells that secreted anti-human interleukin-6 (hIL-6) monoclonal antibodies (SK2 mAb) were microencapsulated within alginate–poly(L)lysine–alginate (APA) membranes (APA-SK2 cells) for immunoisolation, and a single intraperitoneal injection of these APA-SK2 cells remarkably improved IgG1 plasmacytosis in hIL-6 transgenic mice (hIL-6 Tgm). However, the duration of the effectiveness of APA-SK2 cells as a cytomedicine was unfortunately limited. In this study, we attempted to re-inject APA-SK2 cells into hIL-6 Tgm for the purpose of prolonging the cytochemical therapy. In hIL-6 Tgm re-injected with APA-SK2 cells, the plasma IgG1 level did not show any increase in 37 week old mice, and their survival time was at least three times longer than those of untreated hIL-6 Tgm. These results suggest that re-injected APA-SK2 cells survived and secreted SK2 mAb in the allogeneic mice. Thus, the limited duration of the cytochemical effects of APA-SK2 cells was probably caused by the disappearance of the inner space of microcapsules for cell proliferation, not by the rejection of the host's immune system. Therefore, if we can regulate the proliferation of the cells microencapsulated within a semipermeable membrane, we may be able to develop a cytomedicine which will continue its function longer after a single injection.

Key words drug delivery system; cytomedicine; alginate–poly(L)lysine–alginate microencapsulation; SK2 hybridoma cell; human interleukin-6 transgenic mouse

We have attempted the development of cytomedicine, a novel concept which involves using cells as carriers of bioactive molecules. In previous research, we reported that IgG1 plasmacytosis and mesangio-proliferative glomerulonephritis in human interleukin-6 transgenic mice (hIL-6 Tgm) were markedly improved by a single intraperitoneal injection of SK2 hybridoma cells microencapsulated within alginate–poly(L)lysine–alginate membranes (APA-SK2 cells) or agarose gel (Aga-SK2 cells).^{1–3)} Moreover, we confirmed that APA-SK2 cells (BALB/c mice origin) survived and secreted anti-hIL-6 monoclonal antibodies (SK2 mAb) in allogeneic C57BL/6 mice which were the origin of hIL-6 Tgm.²⁾ These results indicated that cytomedicine with a semipermeable membrane had long-term function in bioactive molecules delivery *in vivo* without the immediate rejection of encapsulated cells by the allogeneic host's immune system.

However, we found that the effective duration of cytochemical therapy using APA-SK2 cells was limited.²⁾ We considered the following reasons as possible causes for the limiting of the effectiveness of cytomedicine: a) destruction of microencapsulated cells by the host's immune system which was induced by the injection of cytomedicine, and b) a limited inner space of the microcapsules for cell proliferation. We have previously reported that the APA-SK2 cells could remain viable and proliferate in the allogeneic mice, which had been sufficiently immunized with SK2 cells, as well as in the intact mice.⁴⁾ Moreover, at day 100 after intraperitoneal injection into host we observed that the collected APA microcapsules were filled with SK2 cells and the inner SK2 cells

showed calcification due to over-growth in the microcapsule.³⁾ On the basis of these results, we presumed that the main reason for the reduced effectiveness of APA-SK2 cells in allogeneic mice might be not rejection by the host's immune system, but the limited inner space of the microcapsule for cell proliferation.

We considered that the medical application of cytomedicine using living cells, which perform a drug delivery system meeting targeted temporal, quantitative, and location specifications on hormones, cytokines or antibodies in the living body, can realize the ideal pharmacotherapy for a number of congenital or acquired diseases which need the permanent delivery of bioactive molecules. Consequently, it is desirable that the effective duration of cytomedicine is prolonged as long as possible. As the APA-SK2 cells injected into allogeneic mice were protected from destruction by the host's immune system,⁴⁾ we attempted to prolong the effective duration of cytochemical therapy by re-injecting APA-SK2 cells into hIL-6 Tgm, which were subject to massive IgG1 plasmacytosis with aging. The IgG1 concentration in plasma of hIL-6 Tgm was measured as a parameter in evaluating the therapeutic effect.

MATERIALS AND METHODS

Animals C57BL/6 mice (female, 5 weeks old) were purchased from the Shimizu Experimental Animal Co., Kyoto. The hIL-6 Tgm, which was produced by introducing the hIL-6 cDNA utilizing C57BL/6 mice,⁵⁾ was kindly provided by

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Cells SK2 cells that produce and secrete SK2 mAb⁶⁾ and MH60.BSF2 cells that proliferate in an IL-6 dose-dependent manner⁷⁾ were maintained as previously described.²⁾

Microencapsulation of SK2 Cells within the APA Membrane The general technique of microencapsulation has been described previously.²⁾

Therapeutic Treatment of hIL-6 Tgm Four hIL-6 Tgm (7 weeks old) received an intraperitoneal injection of 3 ml of APA-SK2 cells (5.0×10^6 cells per ml of capsules) in Dulbecco's phosphate-buffered saline (PBS(-)) using an 18-gauge needle. An additional four hIL-6 Tgm between 7 and 29 weeks old received an intraperitoneal injection of 500 μ l of APA-SK2 cells (1.0×10^6 cells per ml of capsules) in PBS(-) every ca. 30 d. Five untreated hIL-6 Tgm or seven littermates which did not have the hIL-6 gene were used as the control group or normal group, respectively. Blood samples were recovered from the tail vein of the mice, and plasma IgG1 concentrations were measured using a mouse IgG1-ELISA.

Measurement of Serum SK2 mAb Concentration in C57BL/6 Mice Injected with APA-SK2 Cells Four C57BL/6 mice, which were the origin of hIL-6 Tgm, were injected with 500 μ l of APA-SK2 cells (1.0×10^6 cells per ml of capsules) in PBS(-). Serum SK2 mAb levels in these mice were measured using the MH60.BSF2 cells-proliferation assay system according to the previous method.²⁾

RESULTS AND DISCUSSION

In our previous report, following intraperitoneal injection into C57BL/6 mice, the viable cell number and SK2 mAb-secretion of collected APA-SK2 cells increased for 21 d and then decreased after day 30 post-injection.²⁾ Moreover, the maximum serum level of SK2 mAb in these allogeneic hosts, which were intraperitoneally injected with APA-SK2 cells (5.0×10^6 cells per ml of capsules, 3 ml), reached about 5 mg/ml.²⁾ On the other hand, it was reported that the serum hIL-6 level in hIL-6 Tgm was 200–400 pg/ml.⁸⁾ Thus, in order to prolong the appropriate therapeutic effect on IgG1 plasmacytosis, we decided to intraperitoneally inject APA-SK2 cells (1.0×10^6 cells per ml of capsules, 500 μ l) into hIL-6 Tgm every ca. 30 d. As shown in Fig. 1, the plasma IgG1 concentration in the untreated hIL-6 Tgm (control group) increased rapidly 9 weeks after birth. In contrast, the increase in the plasma IgG1 concentration was markedly suppressed in hIL-6 Tgm which received a single injection of APA-SK2 cells (APA-SK2 cells group), although the plasma IgG1 concentration in this group began increasing when the mice were over 17 weeks old. This effective duration of APA-SK2 cells was in accordance of the period when SK2 mAb was detected in the serum of C57BL/6 mice injected with APA-SK2 cells.²⁾ In hIL-6 Tgm re-injected with APA-SK2 cells (re-injected APA-SK2 cells group), the plasma IgG1 level did not show any increase by after 37 weeks. This result indicated that the therapeutic effect of APA-SK2 cells on IgG1 plasmacytosis was significantly prolonged by re-injection. Figure 2 shows the effect of the re-injected APA-SK2 cells on survival times of hIL-6 Tgm. The maximum survival time of the control group was 18 weeks. In the APA-SK2 cells group, all mice survived for 27 weeks but died by 30 weeks. The re-injected APA-SK2 cells group all survived

Fig. 1. Therapeutic Effect of Re-injected APA-SK2 Cells on IgG1 Plasmacytosis in hIL-6 Tgm

The IgG1 concentration in plasma was measured using a mouse IgG1-ELISA. (○): Normal mice; (●): untreated hIL-6 Tgm (control group); (Δ): hIL-6 Tgm injected with APA-SK2 cells (5.0×10^6 cells per ml of capsules, 3 ml) at 7 weeks old; (▲): hIL-6 Tgm between 7 and 29 weeks old injected with APA-SK2 cells (1.0×10^6 cells per ml of capsules, 500 μ l) every ca. 30 d. Each data point represents the mean $\pm$ S.E.M. for at least 4 mice.

Fig. 2. Effect of Re-injected APA-SK2 Cells on the Survival Time of hIL-6 Tgm

(●): Untreated hIL-6 Tgm (control group); (Δ): hIL-6 Tgm injected with APA-SK2 cells (5.0×10^6 cells per ml of capsules, 3 ml) at 7 weeks old; (▲): hIL-6 Tgm between 7 and 29 weeks old injected with APA-SK2 cells (1.0×10^6 cells per ml of capsules, 500 μ l) every ca. 30 d.

until at least 39 weeks old. These results demonstrate that the effective duration of cytomedical therapy is markedly prolonged by the re-injection of the cytomedicine; moreover, this also suggests that the re-injected APA-SK2 cells are not rejected by the host's immune system.

In order to confirm that the therapeutic effects of APA-SK2 cells described above depended on the SK2 mAb secreted from APA-SK2 cells, we measured the serum SK2 mAb concentration in C57BL/6 mice injected with 500 μ l capsules of APA-SK2 cells (Fig. 3). After a single injection of APA-SK2 cells, the serum SK2 mAb level rapidly increased, approximating the results seen in C57BL/6 mice injected with 3 ml capsules of APA-SK2 cells,²⁾ and by day 28 the level reached 400 μ g/ml. Until day 42 post-injection, we detected SK2 mAb in serum. On the basis of our previous immunological study,⁴⁾ the re-injected APA-SK2 cells could probably secrete as much SK2 mAb as the APA-SK2 cells which were initially injected. In this experiment, we presumed that the serum SK2 mAb level might be between 200 and 400 μ g/ml for treatment with re-injection of APA-SK2 cells. Thus, following re-injection of 500 μ l capsules of APA-SK2 cells every ca. 30 d, the SK2 mAb level in serum was kept at a sufficiently high concentration to demonstrate a therapeutic effect on IgG1 plasmacytosis. Although we tried to evaluate the change of serum hIL-6 level in hIL-6 Tgm

Fig. 3. Serum SK2 mAb Level in C57BL/6 Mice Injected with APA-SK2 Cells

500 μ l of APA-SK2 cells (1.0×10^6 cells per ml of capsules) were intraperitoneally injected into normal C57BL/6 mice. A solid line represents the serum SK2 mAb concentration which was measured by MH60.BSF2 cells-proliferation assay system. Each data point represents the mean $\pm$ S.E.M. of 4 mice. The days when APA-SK2 cells were re-injected into hIL-6 Tgm are indicated by arrows. The predicted serum SK2 mAb level after the second injection of APA-SK2 cells is indicated by the dotted lines.

which were intraperitoneally re-injected with APA-SK2 cells, it could not be detected (data not shown). We surmise that hIL-6 Tgm re-injected with APA-SK2 cells had enough SK2 mAb in the serum for neutralization of free hIL-6.

When cytomedicine is applied to an allogeneic host, the effective duration of cytomedicine can be prolonged by re-injection similar to conventional drugs. However, in this study there is the serious problem that the re-injected APA-SK2 cells may accumulate in the host's body after lost of function as cytomedicine. In consideration of the characteristics of cytomedicine, we need to develop a cytomedicine which functions as a permanent carrier of bioactive molecules by a single injection. We had confirmed in a preliminary experiment that APA microcapsules remained intact without biodegradation for at least one year *in vivo*. If we can microencapsulate living cells whose proliferation is regulated without compromising their cellular metabolism and function, then the ideal cytomedical therapy will be realized. The ways in which we can regulate the cell-growth include using cells; a) which demonstrate density-dependent growth arrest,⁹⁾ b) which are

cultivated as spheroids,¹⁰⁾ and c) which are highly differentiated.¹¹⁾ In the future, cells whose growth rate can be controlled should be microencapsulated within a semipermeable membrane.

In addition, the cells in a living body have the original control system that consists of functions for the biosynthesis, sensor, and controlled release of bioactive molecules. If we can also incorporate these functions into a cytomedicine, it will bring about a significant step forward in pharmacotherapy.

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