1. Introduction

Dendritic cells (DCs) are found in most tissues, and they capture and process antigens and display large amounts of MHC-peptide complexes on their surfaces (Banchereau and Steinman, 1998; Banchereau et al., 2000). Because DCs display both MHC-class II molecules and co-stimulatory molecules such as CD80 and CD86, only DCs can induce primary sensitization against specific antigens in naïve T cells (Lanzavecchia and Sallusto, 2001). It is generally accepted that dendritic cell-like cells (monocyte-derived dendritic cells, Mo-DCs) are induced from peripheral blood mononuclear cells (PBMCs) by granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) in vitro (Sallusto and Lanzavecchia, 1994). Mo-DCs have many features similar to those of primary DCs, including antigen capture, co-expression of co-stimulatory molecules with MHC molecules, and secretion of IL-12 (Cella et al., 1997). It has been suggested that Mo-DCs, injected intradermally or subcutaneously, capture and process antigens, move to the T cell-dependent areas of secondary lymphoid organs, and stimulate naïve T cells (Thomas et al., 1999; Thurner et al., 1999). In this capacity, Mo-DCs have been utilized as vectors for vaccine therapies against various cancers (Dallal and Lotze, 2000; Fong and Engleman, 2000; Banchereau et al., 2001; Reinhard et al., 2002; Schuler et al., 2003).

Several problems must be addressed to evaluate DC function for vaccine therapy. First, most data concerning the antigen-presenting ability of Mo-DCs have been obtained with PBMCs from healthy donors, not cancer patients. Recent studies have indicated that Mo-DCs from advanced cancer patients are impaired at several stages of the antigen-presenting process (Onishi et al., 2002), suggesting that the antigen-presentation-related functions of individual Mo-DCs used for vaccine therapies must be evaluated closely. A second problem is that very little is known about the biological behaviors of Mo-DCs administered to cancer patients.

Three-dimensional (3-D) hydrated collagen lattices have been widely used for in vivo-like culture of various types of cells including tumor cells, lymphoid cells, and DCs (Friedl et al., 1993, 1995; Gunzer et al., 1997; Nakamura et al., 2002) because many studies have shown that the fiber distribution and biophysical architecture of collagen lattices closely resemble interstitial soft tissues, dermis, and network-like stroma of the lymph node (Friedl et al., 1998; Friedl and Brocker, 2000; Gunzer et al., 2000b). For example, Gunzer et al. (1997) developed a unique method to analyze individual DC migration within a 3-D collagen lattice. However, these complicated methods are difficult for evaluating the antigen-presenting ability of Mo-DCs used in DC-vaccine therapies.

Mo-DCs are characterized by a high rate of antigen uptake in the immature state and high antigen-presenting function (surface marker and cytokine production) in the mature state. To capture antigens, Mo-DCs have to migrate toward antigens. In addition, antigen-capturing Mo-DCs mature, then move to the T-dependent areas of secondary lymphoid organs, and stimulate naïve T-cells. In conventional monolayer culture system, we must evaluate various kinds of Mo-DC's functions by an individual method. We describe here a novel method to evaluate the quality of Mo-DCs used for cancer therapy. Our 3-D model consists of two collagen gel layers: a lower layer containing Mo-DCs and an upper layer containing necrotic tumor cells or necrotic tumor cells and T cells. In our model, GCTM-1-capturing Mo-DCs mean that Mo-DCs moved toward necrotic GCTM-1 and captured them. And increase of surface marker such as HLA-DR and CD80 indicates maturation of Mo-DCs. Increase of IL-12 production by Mo-DCs indicates activation of Mo-DCs. In addition, IFN-γ production by CD4+ T cells indicates ability of CD4+ T cell activation by Mo-DCs. By our two-layer culture system, we can estimate various kinds of Mo-DC's functions at a time. Interestingly, phase-contrast microscopy allowed us to analyze several Mo-DCs functions, including migration, antigen capture, phagocytosis, and cytokine secretion, in this system in real time. In the future, video microscopy may allow us to analyze in vivo-like behaviors of Mo-DCs.

2. Materials and methods

2.1. Generation of Mo-DCs

Mo-DCs were generated from the adherent fraction of PBMCs of healthy volunteers as previously de-

scribed but with minor modifications (Kuppner et al., 2001). In brief, PBMCs were isolated from heparinized peripheral blood by Histopaque-1077 (Sigma, St. Louis, MO) density gradient centrifugation. PBMCs were resuspended in RPMI 1640 basal medium (Sanko Pure Chemicals, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Filtron, Australia), 100 μg/ml penicillin (Meijiseika, Tokyo, Japan), and 100 μg/ml streptomycin (Meijiseika), plated at a density of 2×10^6 cells/ml, and allowed to adhere in 24-well culture plates (Nalge Nunc International, Chiba, Japan) overnight at 37 °C. The nonadherent cells were then removed, and the adherent cells were harvested and cultured in 10% FBS-containing RPMI. GM-CSF (500 ng/ml) (Novartis Pharma Basel, Switzerland) and IL-4 (500 U/ml) (Ono, Tokyo, Japan) were added on day 0. On day 7, cultured cells were collected as immature Mo-DCs. Immature Mo-DCs were further purified by negative selection with magnetic beads coated with mouse monoclonal anti-CD2, anti-CD3, and anti-CD19 antibodies (Dynabeads, Dynal, Oslo, Norway) as previously described but with minor modifications (Vartdal et al., 1987). This depletion procedure yielded greater than 90% CD14 −, CD80+, HLA-DR+ immature Mo-DCs.

2.2. CD4+ T cells

CD4+ T cells were purified from fresh human PBMCs with a CD4+ isolation kit (Dynabeads, Dynal) according to the manufacturer's instructions. The purity of CD4+ T cells was greater than 98% as analyzed with a FACS Calibur flow cytometer and CELLQuest software (Becton Dickinson, San Jose, CA).

2.3. Tumor cells and induction of necrosis

Human gastric carcinoma cell line GCTM-1 was maintained in 10% FBS-containing RPMI at 37 °C. Necrotic GCTM-1 cells were induced as previously described but with minor modifications (Nestle et al., 1998). In brief, tumor cells were washed with phosphate-buffered-saline (PBS) (Wako, Osaka, Japan) and then resuspended in RPMI. Cells were lysed by five cycles of freezing in liquid nitrogen and thawing at 37 °C. Lysis was monitored by light microscopy. Larger particles were removed by centrifugation. Whole cell lysates were mixed with collagen gel as tumor-associated antigens.

2.4. 3-D two-layer collagen gel culture model

Mo-DCs were used at 4×10^5 cells per assay and suspended in 20-μl RPMI containing with 1% human serum albumin (HSA). Mo-DCs were mixed with an equal volume of chilled type I collagen (Kokencellgen I-AC: 0.3%) (Funakoshi, Tokyo, Japan). The final collagen concentration was 0.15%. The mixture was transferred to 96-well plates (Nalge Nunc International) at 40 μl/well. Before the mixture was allowed to polymerize, a mixture of 20-μl RPMI with 1% HSA containing 4×10^5 necrotic GCTM-1 cells mixed with an equal volume of chilled type I collagen was layered onto the Mo-DC mixture. The two layers of mixture were then allowed to polymerize for approximately 1 h at 37 °C. After polymerization, 200-μl RPMI with 1% HSA was added to each well. A schematic of our culture model is shown in Fig. 1. These 3-D two-layer cultures were then incubated at 37 °C in a humidified atmosphere of 5% CO_2. Liquid culture medium was changed everyday and stored at −80 °C for later analyses. Mo-DCs suspended in the collagen matrix were observed by phase-contrast microscopy. Microscope was connected with digital camera, COOLPIX 950 (Nikon, Tokyo, Japan) and images were recorded in XGA-size (1024 × 768 pixels).

2.5. Time-lapse videomicroscopy

Dynamic cell motility was recorded as follows. Cells embedded within the collagen gel matrix were visualized on a phase-contrast microscope. The image was monitored on a screen of 15 inches monitor, 15ZR7 (Toshiba, Tokyo, Japan) and recorded with a digital video recorder, DCR-PC9 (Sony, Tokyo, Japan). We could focus on individual cells consecutively and also record time-lapse movements of individual cells. Furthermore, we could select certain images at will for further examination.

2.6. Cell viability

To evaluate cell viability, Mo-DCs existing in gel were stained with DNA-binding fluorochrome bis-

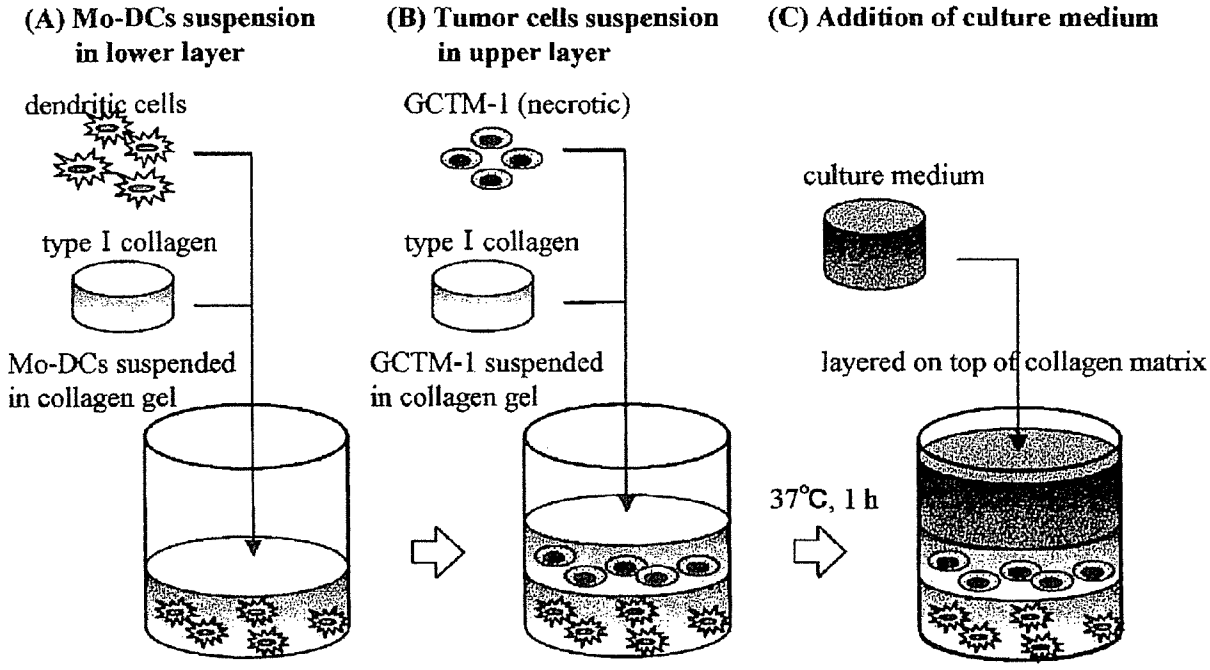

Fig. 1. Schematic of 3-D two-layer collagen matrix culture model. (A) Mo-DCs were suspended in 20-μl RPMI 1640 with 1% HSA. and mixed with an equal volume of chilled type I collagen. The mixture was transferred to 96-well plates at 40 μl/well. (B) Before the mixture containing Mo-DCs was allowed to polymerize, collagen gel containing tumor cells (made in the same way as the Mo-DCs mixture) was layered onto the DC mixture. (C) Two-layer collagen matrix was allowed to polymerize for approximately 1 h at 37 °C. After polymerization, 200-μl culture medium was added. This 3-D two-layer culture model was incubated at 37 °C in a humidified atmosphere of 5% CO_2 and observed with time-lapse microscopy.

benzimide stain (Hoechst 33342; Molecular Probes, Eugene, OR) and propidium iodide (PI; Molecular Probes). Briefly, 4×10^5 Mo-DCs were cultured in the mixture of RPMI with 1% HSA and type I collagen using 96-well plastic plate. Twenty microliters of Hoechst 33342 was added to culture medium at first and then incubated for 1 h at 37 °C. Next, 20 μl of PI was added to culture medium and incubated for 10 min at 37 °C. After these incubation times, fluorescence-positive cells were counted with a fluorescence microscope. Hoechst-positive and PI-negative cells were considered viable and PI-positive cells were considered nonviable. We calculated the ratio of a fluorescence-dyeing cell for 100 cells. Data were expressed as the mean ± S.D. of percent fluorescence-positive cells of eight independent wells.

2.7. Capture of necrotic GCTM-1 cells by Mo-DCs

Mo-DCs and necrotic GCTM-1 were labeled with PKH67 (green) and PKH26 (red) fluors (Sigma), respectively. Fluorescently-labeled Mo-DCs and necrotic GCTM-1 cells were embedded separately into collagen matrix and then observed by fluorescence microscopy. When images of Mo-DCs were superimposed with those of GCTM-1 cells, areas of co-localization appeared yellow or orange. Such cells were considered necrotic tumor-capturing Mo-DCs. Mo-DCs in the upper layer (migrating Mo-DCs) were counted in five upper fields (x200) at random. Percent phagocytosis represents the ratio of a tumor-capturing Mo-DC for total migrating Mo-DCs. Data were expressed as the mean ± S.D. of percent phagocytotic Mo-DC.

2.8. Collection of cells from 3-D two-layer collagen gel culture model

Mo-DCs were harvested from the collagen matrix by digestion with collagenase (Wako) as previously described but with minor modifications (Friedl et al., 1995). In brief, collagen matrix containing Mo-DCs was incubated with highly purified collagenase (final

concentration, 7500 U/ml) for 5 min. Cells were washed two times with PBS (Wako) and resuspended in RPMI for further study.

2.9. Expression of antigen-presentation-related molecules on Mo-DCs

To analyze the expression of antigen-presentation-related molecules on Mo-DCs, cells collected from collagen matrix were incubated for 1 h with one of the following monoclonal antibodies (BD Pharmingen, San Diego, CA) conjugated to FITC for direct staining: anti-CD83, anti-HLA-DR, or PE-anti-CD80, PE-anti-CD86. The isotype controls, IgG1 and IgG2, were also obtained from BD Pharmingen. For staining, cells were washed two times with PBS (Wako) and incubated in PBS containing 3% bovine serum albumin (BSA) (Sigma) and 0.1% NaN_3 (Sigma) (referred to as FACS buffer) and the appropriate concentration of labeled mAb for 1 h at 4 °C. After cells were washed with FACS buffer, the fluorescence intensities of gated Mo-DCs populations were measured with a FACS Calibur flow cytometer and analyzed with CELLQuest software (Becton Dickinson).

2.10. IL-12 and interferon-γ (IFN-γ) secretion

Culture supernatants were collected every 24 h and the concentrations of IL-12 p40 and interferon-γ (IFN-γ) were determined by enzyme-linked immunosorbent assay (ELISA) kits specific for IL-12 p40 and IFN-γ (Biosource, Camarillo, CA). ELISA protocols were described previously (Wilkinson et al., 1996). The detection limit of these ELISAs for IL-12 p40 is 2 pg/ml and for IFN-γ is 4 pg/ml.

3. Results

3.1. Morphology of Mo-DCs

Mo-DCs embedded in collagen gels (lower layer) were observed by phase-contrast microscopy. In collagen lattices, Mo-DCs extended processes from the cell

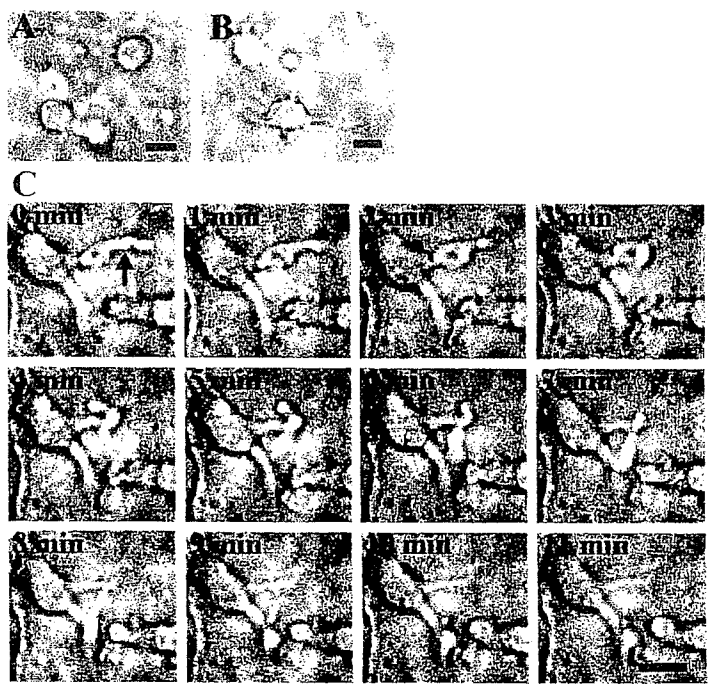

Fig. 2. Appearance of Mo-DCs in 3-D collagen matrix. Mo-DCs were cultured in collagen matrix for 7 days and were observed under phase-contrast microscopy. (A) Some Mo-DCs had a round shape right after cultivation in collagen matrix. (B) Most Mo-DCs extended processes in many directions on day 4. (C) Time-lapse analysis of dendrite formation by Mo-DC in collagen matrix. The formation of dendrites on day 4 was observed using time-lapse videomicroscopy. Dendrite (black arrow) showed continuous movement during observation. Images are shown at 1-min intervals. The time of the first image is arbitrarily set to 0 min. Magnification, ×400. Scale bars, 10 μm.

membrane that were characteristic within 24 h after initial culture. As shown in Fig. 2A, some Mo-DCs retained a round shape with many short processes. Some Mo-DCs changed to a spherical shape and extended several long "dendritic" processes that were 20- to 50-μm long (Fig. 2B).

Videomicroscopy revealed that Mo-DCs embedded in collagen gels developed considerable flexibility in cell shape. Dendrites on cell bodies repeatedly extended and retracted the processes/dendrites over the course of several minutes (Fig. 2C).

3.2. Survival of Mo-DCs

Immature Mo-DCs were generated from PBMCs by culture in medium containing GM-CSF and IL-4 for 7 days as described in Materials and methods. These immature Mo-DCs were embedded within collagen gel and cultured in RPMI containing 1% HSA for several weeks. Culture medium was changed every 24 h. Cells were observed by phase-contrast microscopy. On day 7, many Mo-DCs maintained multiple short processes or several long processes (Fig. 3A) and these cells seemed to be viable. As culture periods were extended, however, the number of Mo-DCs showing cellular fragmentation increased gradually (Fig. 3B). These Mo-DCs were considered as dead cells, probably apoptotic cells. To evaluate cell viability, Mo-DCs were stained with Hoechst 33342 and PI and evaluated with a fluorescent microscope as described in Materials and methods. Cells were alive until 7 days after the initial culture (day 7) and viable cells decreased gradually after day 9 (Fig. 3C, left panel). The percentage of viable cells on day 15 was $40.2 \pm 6.91\%$. On the other hand, nonviable cells increased gradually after day 9 and reached $59.6 \pm 6.12\%$ on day 15 (Fig. 3C, right panel). Most Mo-DCs appeared to be dead by day 21. The data are representative of five independent experiments using Mo-DCs generated from three different donors.

3.3. Migration and phagocytosis of Mo-DCs

When Mo-DCs and necrotic GCTM-1 were embedded in collagen gel (single-layer collagen gel culture), approximately 10% of Mo-DCs were active and migrated over a distance of 10 μm. Time-lapse videomicroscopy showed a Mo-DC migrating toward a necrotic GCTM-1 fragment that was about 30 μm away (Fig. 4). The Mo-DC then migrated to the GCTM-1 fragment, made contact with it, and engulfed it (phagocytosis) within 10 min.

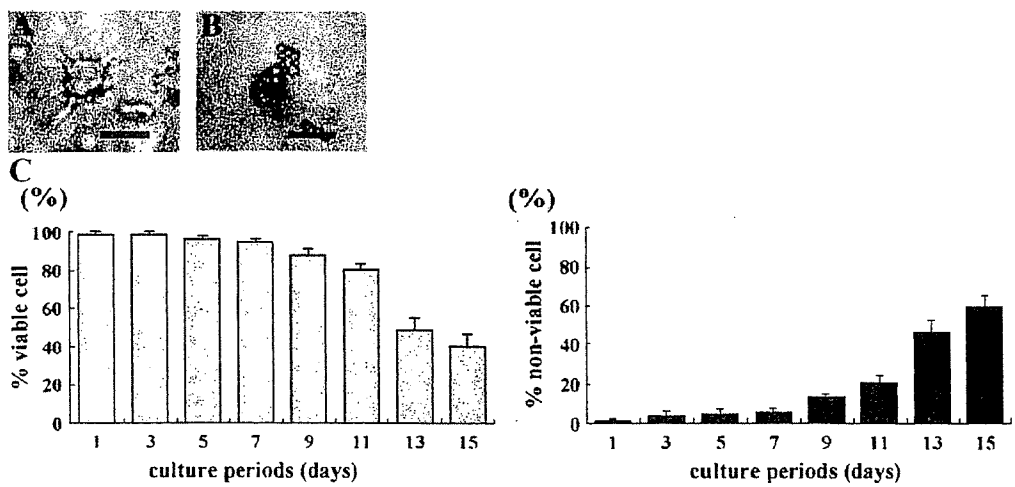

Fig. 3. Survival of Mo-DCs. Mo-DCs were cultured in collagen matrix and were observed under phase-contrast microscopy and fluorescence microscopy. (A) On day 7, most Mo-DCs had some processes and seemed to be still alive. Magnification, ×400. Scale bars, 10 μm. (B) On day 14, many Mo-DCs suddenly appeared fragment. These cells seemed to be dead. Magnification, ×400. Scale bars, 10 μm. (C) Cell viability was determined with Hoechst 33342 and PI staining as described in Materials and methods. Hoechst-positive and PI-negative cells were considered viable and PI-positive cells were considered nonviable. The data are representative of five independent experiments using Mo-DCs generating from three different donors. Data were expressed as the mean ± S.D. of percent fluorescence-positive cells of eight independent wells.

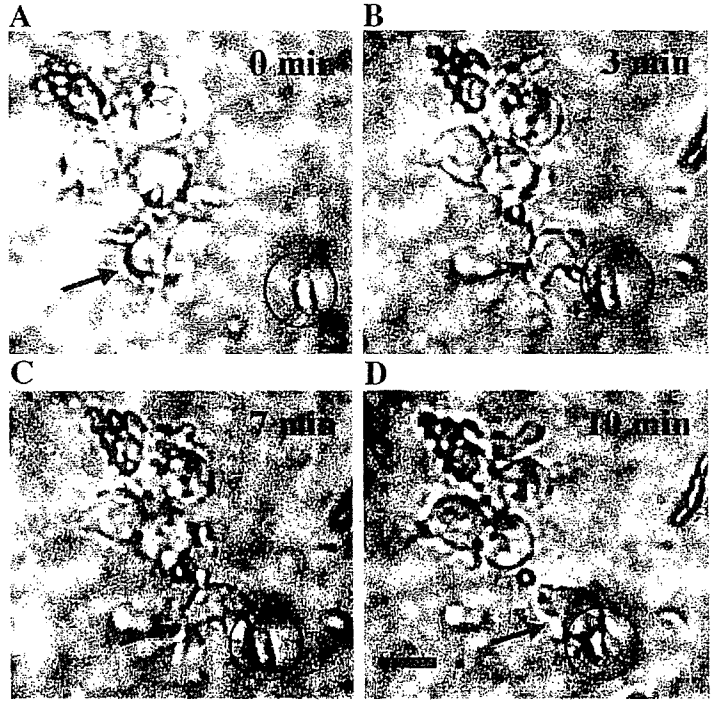

Fig. 4. Migration of and phagocytosis by Mo-DC in 3-D collagen gel culture. The Mo-DC migrated toward a necrotic tumor cell and phagocyted it over a time course of 10 min. (A) A cluster of Mo-DCs is visible in collagen matrix. (B, C) One (black arrows) moved spontaneously toward a fragment of necrotic GCTM-1 cell (black circle). (D) Ten minutes after initial observation, the Mo-DC reached the fragment and internalized it. Magnification, ×400. Scale bars, 10 μm.

With our two-layer collagen gel model, we were able to evaluate simultaneously both migratory and phagocytic abilities of Mo-DCs. For this purpose, Mo-DCs were labeled with the green fluorescent marker PKH67 and necrotic GCTM-1 were labeled with the red fluorescent marker PKH26. Fluorescently labeled cells were embedded separately in collagen gels. The lower collagen gel layer contained Mo-DCs (green), and the upper layer contained necrotic GCTM-1 (red). We then imaged this 3-D two-layer model by fluorescence microscopy (Fig. 5). On day 1 of culture, many Mo-DCs migrated from the lower layer to the upper layer, and some had engulfed necrotic GCTM-1 (yellow). In the first 24 h of incubation, 10% to 20% of Mo-DCs migrated into the upper layer. Percent phagocytosis was $13.2 \pm 3.03\%$ (Fig. 5E). Then the number of migrating Mo-DCs decreased after 24 h. In contrast, percent phagocytosis increased to $19.4 \pm 4.72\%$ at day 2 (Fig. 5E). The data are representative of three independent experiments using Mo-DCs generated from three different donors.

3.4. Expression of antigen-presentation-related molecules on Mo-DCs

Mo-DCs were embedded in collagen gel with or without necrotic GCTM-1. Seven days after initiation of culture, Mo-DCs were collected from collagen gels with collagenase, and the expression of antigen-presentation-related molecules such as HLA-DR, CD80, and CD86 was examined with FACS analysis. Mo-DCs cultured with necrotic GCTM-1 showed increased expression of HLA-DR, CD80, and CD86 in comparison with Mo-DCs cultured in the absence of necrotic GCTM-1 (Fig. 6). The data are representative of three independent experiments using Mo-DCs generated from three different donors.

3.5. IL-12 secretion by Mo-DCs

Using our 3-D two-layer collagen gel model, we investigated secretion of IL-12 and IFN-γ by Mo-DCs. Culture medium was changed every day and stored at -80 °C until assay. The concentration of

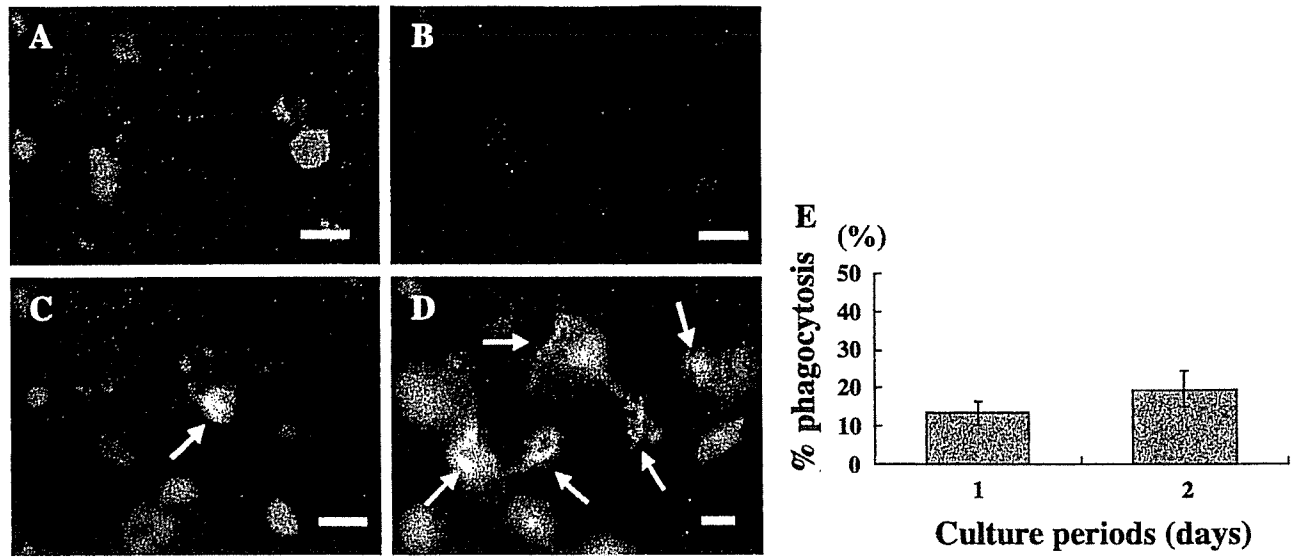

Fig. 5. Migration and phagocytosis of Mo-DCs in 3-D two-layer collagen matrix. This model consisted of two different layers of collagen gels. (A) The lower layer contained Mo-DCs labeled with PKH67 (green). (B) The upper layer contained necrotic GCTM-1 cells labeled with PKH26 (red). (C, E) Twenty-four hours after the initial cultivation (day 1), Mo-DCs migrated to the upper layer and were seen at the same field with tumor cells. Some of them appeared to be yellow cells (white arrow), implying phagocytosis of tumor cells by Mo-DCs. The percentage of phagocytosis on day 1 was 13.2 ± 3.03%. (D, E) Forty-eight hours after the initial cultivation (day 2), the number of Mo-DCs that had engulfed necrotic GCTM-1 had increased. The percentage of phagocytosis on day 2 was 19.4 ± 4.72%. Data represent the mean ± S.D. of five experiments. A representative experiment of three is shown. Magnification, ×200. Scale bars, 10 μm.

each cytokine represents roughly the daily secretion of each cytokine. When the upper layer contained necrotic GCTM-1, Mo-DCs secreted large amounts of IL-12 between days 3 and 5. Secretion of IL-12 decreased gradually from days 6 (Fig. 7A). To exclude a possibility that GCTM-1 themselves release IL-12, necrotic GCTM-1 alone were cultured in collagen matrix for 7 days. No IL-12 was detected in this culture condition. When the upper layer did not contain necrotic GCTM-1, IL-12 secretion was not detected during the 7 days of culture. IFN-γ was not detected regardless of the presence of necrotic GCTM-1 in the upper layer (data not shown).

When the upper layer contained both necrotic GCTM-1 and CD4+ T cells, the pattern of IL-12 secretion was very similar to that when the upper

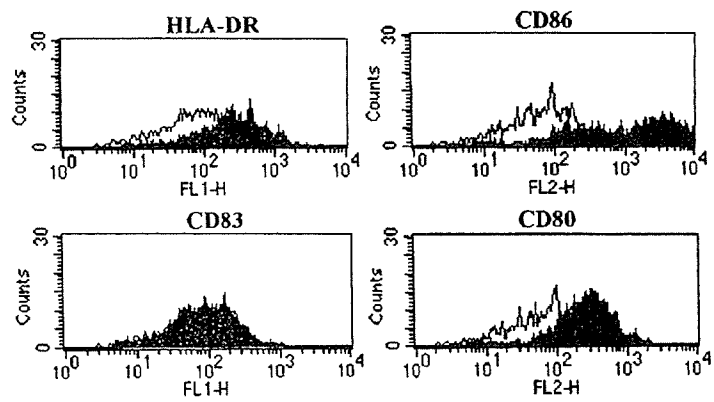

Fig. 6. Expression of antigen-presentation-related molecules on Mo-DCs in 3-D collagen gel. Cells were collected and analyzed on day 7. Mo-DCs cultured with necrotic GCTM-1 (shaded curves) showed increased expression of HLA-DR, CD86, and CD80 compared with Mo-DCs cultured without necrotic GCTM-1 (solid curves). The data are representative of three independent experiments using Mo-DCs generated from three different donors.

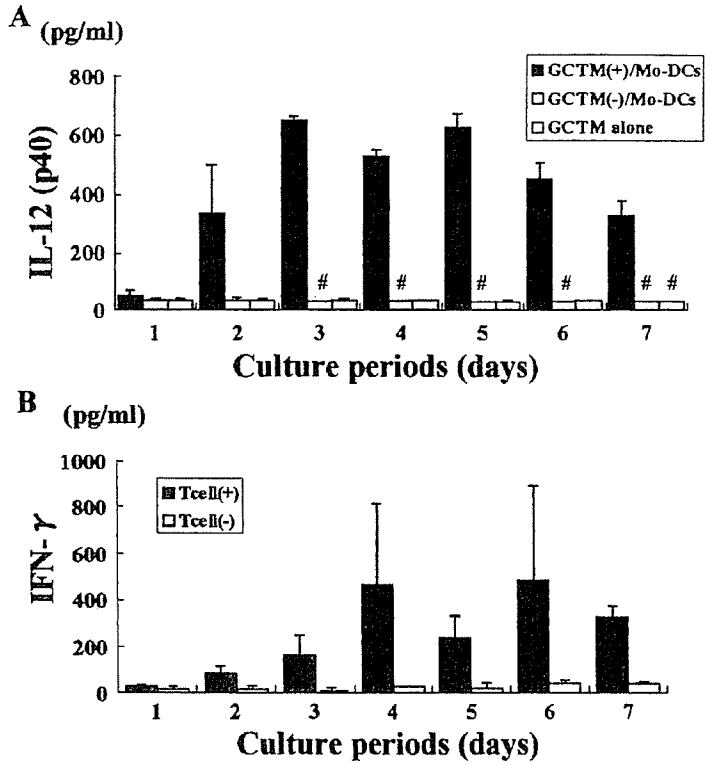

Fig. 7. Cytokine secretion by Mo-DCs in 3-D two-layer culture model. The supernatants were collected daily from 3-D two-layer collagen gel culture that contained Mo-DCs in the lower layer. (A) When the upper layer contained necrotic GCTM-1 [GCTM(+)/Mo-DCs], IL-12 p40 secretion was detected from days 1 to 7. In contrast, IL-12 p40 secretion was barely detected when the upper layer lacked necrotic GCTM-1 [GCTM(−)/Mo-DCs]. When the lower layer did not contain Mo-DCs [GCTM alone], IL-12 p40 secretion was not detected. (B) [T cell(−)] represents that the upper layer contains necrotic GCTM-1 alone. [T cell(+)] represents that the upper layer contains both necrotic GCTM-1 and CD4+ T cells. Cytokine concentration represents mean ± S.D. of three wells. The data are representative of three independent experiments using Mo-DCs generated from three different donors. This figure was obtained from a simultaneous experiment using Mo-DCs generated from the same donor.

layer contained only necrotic GCTM-1 (data not shown). Under this culture condition, IFN-γ secretion was detected and continued until at least day 7 (Fig. 7B). The data are representative of three independent experiments using Mo-DCs generated from three different donors. Fig. 7 was obtained from a simultaneous experiment using Mo-DCs generated from the same donor.

3.6. Dynamics of the interaction between Mo-DCs and T cells

As described above (Fig. 2), Mo-DCs embedded within collagen gels showed considerable flexibility in cell shape. Using time-lapse videomicroscopy, we observed individual interactions between Mo-DCs and T cells.

4. Discussion

We describe here results from our experiments with a novel 3-D two-layer collagen gel culture system that can be used to evaluate and monitor Mo-DCs used as vectors for DC-vaccine therapies. With this model system, we were able to evaluate simultaneously multiple functions of Mo-DCs, including migration, phagocytosis of necrotic tumor cells, and interactions with T cells, in real time with phase-contrast or fluorescence microscopy. In addition, we could also observe dynamic cell–cell interactions on a single-cell basis with time-lapse videomicroscopy.

Recent advances in medical technology have made it possible to develop 3-D DC culture systems that are similar to in vivo environments. Hydrated

collagen gel has been used in 3-D cell culture systems, because the structure of collagen lattices resembles that of several tissues, including dermis and the network-like stroma of lymph node (Friedl and Brocker, 2000). Gunzer et al. (1997, 2000a,b) have reported data that support the usefulness of 3-D collagen matrix systems in the study of in vivo-like DC behaviors, especially migration and DC–T cell interactions. Murine epidermal Langerhans cells and bone marrow DCs were used as target cells and embedded in collagen lattices for analysis on an inverted confocal scanning microscope. Gunzer et al. (1997, 2000a,b) showed that DCs formed extended membrane processes, which are characteristic of these cells. They recently described a modified method by which migration of murine DCs within 3-D collagen lattices can be analyzed by time-lapse videomicroscopy and computer-assisted single-cell tracking. These in vitro models certainly inform us of missing in vivo behavior of DCs. In the present study, we focused on the development of a method that allows easy and simultaneous evaluation of multiple functions of Mo-DCs. It is generally accepted that DCs play many roles in induction of specific T cell immunity in vivo. It is thought that DCs migrate to, capture, and then process antigens, move to T cell-dependent areas of secondary lymphoid organs, and stimulate naïve T cells (Banchereau and Steinman, 1998; Banchereau et al., 2000). On the basis of these functions, we developed a new 3-D two-layer collagen gel culture system. Each layer is almost 1.2-mm thick. In our system, Mo-DCs and necrotic tumor cells exist separately in different collagen gel layers. To capture necrotic GCTM-1, Mo-DCs must migrate to the upper layer. If the upper layer contains T cells, migrating Mo-DCs can make contact with T cells in the upper layer. In our system, 10% to 20% of Mo-DCs migrated from the lower layer to the upper layer within 24 h of incubation, and more than half of the migrating Mo-DCs engulfed necrotic GCTM-1 by day 2 (Fig. 5). Consistent with data from experiments with 2-D culture systems (Labeur et al., 1999; Hochrein et al., 2000; Liu, 2001; Schnurr et al., 2001), we observed in our 3-D culture system that Mo-DCs that capture GCTM-1 secrete high level of IL-12 (Fig. 7A). When the upper layer did not contain necrotic GCTM-1, IL-12 was not detected in culture media. In addition, IL-12 secretion was detected after but not before Mo-DCs appeared in the upper layer (data not shown). Because IFN-γ production was detected only when the upper layer contained CD4+ T cells, IFN-γ may be secreted primarily by CD4+ T cells (Fig. 7). Since it has been shown that IL-12 can induce IFN-γ production in CD4+ T cells (Gerosa et al., 1996; Kuroki et al., 2003), we examined if IL-12 secreted from GCTM-1-capturing Mo-DCs induces IFN-γ in CD4+ T cells existing in the upper layer of our model. As shown in Fig. 7B, IFN-γ secretion occurred only when CD4+ T cells coexisted with necrotic GCTM-1 in the upper layer. In addition, IFN-γ secretion was always followed by IL-12 secretion (Fig. 7A), CD4+ T cells alone in the upper layer or Mo-DCs alone in the lower layer did not produce detectable IFN-γ (data not shown). These results suggest that Mo-DCs migrate toward necrotic GCTM-1, captured them, and secreted IL-12 and that Mo-DC-secreting IL-12 induced IFN-γ in CD4+ T cells.

In 3-D one-layer culture systems, investigators observed that Mo-DCs survive at least 2 weeks within collagen lattices and that Mo-DCs that captured necrotic GCTM-1 showed increased the expression of HLA-DR, CD80, and CD86, which are important for antigen presentation and stimulation of naïve T cells (Liu, 2001; Mellman and Steinman, 2001; Thery and Amigorena, 2001). Expression of these molecules was elevated even on day 7 of incubation (Fig. 6).

In conclusion, using this 3-D two-layer collagen matrix model, we could easily evaluate a series of in vivo-like Mo-DC functions in the antigen presentation process in real time. In addition, we could observe cell interactions on an individual basis by time-lapse videomicroscopy. We are now beginning to use this two-layer collagen gel system for evaluation of the quality of and monitoring of Mo-DCs.

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癌免疫細胞療法における凍結血漿の使用に関する調査研究

平成16年度～平成17年度　総合研究報告書（2/2）

平成18年（2006）4月

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総説

免疫学を基盤とした腫瘍制御法の開発

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要旨　細胞傷害性T細胞の誘導が可能な腫瘍関連抗原の存在が，主として *in vitro* の系において明らかになった。加えて，樹状細胞類似の高い抗原提示能を有する細胞を末梢血単球から誘導する技術も開発された。これらが相まって，種々の癌に対するワクチン療法が始まった。事実，ある種の癌細胞は生体において非自己としての特性を示し，患者のT細胞がこれらの癌細胞と反応する能力を有することもわかってきた。しかしながら，われわれは，「腫瘍細胞は非自己としての特性は有しつつも，先天免疫系が癌細胞を生体にとって危険なものと認識しないために，結果として癌細胞は免疫寛容の世界に存在している」と考えている。もし，われわれの仮説が正しいならば，癌細胞に対する特異的な免疫反応を誘導するには，先天免疫系が癌細胞を細菌やウイルスのように危険なものと認識するように先天免疫系を操作，改変する必要がある。これらの視点に基づいて，新たなワクチン療法として“免疫監視機構構築療法”を開発，実施してきた。本療法は非常に複雑であり，一般的な治療法としての社会貢献は小さいかもしれない。しかし，われわれは，基礎免疫学の理論にできるだけ忠実な治療戦略の開発こそ価値ある癌に対する免疫療法の扉を開けることができると信じている。ここでは，免疫監視機構構築療法を含む現在進行中の免疫学を基盤としたわれわれの腫瘍制御法を紹介する。

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Development of Therapeutic Strategies Based on Immunology against Tumors

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Summary

Tumor associated antigens that are able to induce cytotoxix T cells were identified mainly *in vitro* systems. In addition, recent technology has made it possible to generate dendritic cell-like cells having stronger antigen presentation ability from peripheral blood monocytes. As a result, vaccine-based therapies for various kinds of tumors have been started. In fact, some of the tumors display a “non-self” characteristic *in vivo*, and patients' T cells have the ability to react to these tumor cells. However, we speculate that tumor cells are in the world of immune tolerance because tumor cells are not judged to be dangerous by innate immunity. If our hypothesis is correct, to induce specific immunity against tumor cells, we have to modify the system so that the innate immune system recognizes that tumor cells are dangerous, similiar to bacteria or viruses. Based on these aspects, we developed a new vaccine-based therapy, i.e., immune surveillance architecture therapy. Since this therapy itself is too complicated, it may not make a very large social contribution as a general therapy. We believe a treatment strategy that obeyes the theory of basic immunology as much as possible will open a door to valuable immunotherapy against tumors. In this minireview, we introduce our ongoing studies, including immune surveillance architecture therapy, concerning therapeutic strategies based on immunology against tumors.

Key words: Tumor, Immune surveillance, Immune tolerance, Danger signal, Vaccine therapy
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はじめに

細胞傷害性T細胞（CTL）を誘導し得る腫瘍関連抗原が同定され，さらに強力な抗原提示能を有する樹状細胞様細胞が末梢血単球から誘導可能となり，種々の癌に対するワクチン療法が一斉にスタートした。実に，様々な方法が試みられているが，これらの情報に関してはPubMedを開いてほしい。ここでは，われわれ腫瘍制御学分野において研究開発型医療として開発中の免疫学を基盤とした腫瘍制御法に焦点を絞って紹介する。

I. 感染免疫の概要

免疫系は，生体にとって危険かどうかといったファジー（特異性が低い）なアナログ方式の自然免疫系と自己［0］，非自己［1］といった厳密なデジタル方式（特異性が高い）で組み立てられている獲得免疫に区別して論じられることが多かった。したがって，どちらのシステムを動かすかによって，非特異的免疫療法あるいは特異的免疫療法と呼ばれてきた。しかし，最近の分子生物学的研究成果は，自然免疫系と獲得免疫系が樹状細胞を中心とする抗原提示細胞によりつながっており，自然免疫系が危険と察知しなければ情報が正しく獲得免疫系に伝わらないことを明らかにした。つまり，厳格な獲得免疫系はファジーな自然免疫系のコントロール下にあると理解することができる。生体にとって危険かどうかは，細菌などの抗原侵入を察知したマクロファージや樹状細胞あるいはNK細胞といった自然免疫系細胞が産生するサイトカインの種類によってしばしば判断される。

たとえば，ウイルス感染の場合を想定してみよう。ウイルスが感染した細胞はIFN-αを産生する。IFN-αはNK細胞を活性化し，活性化したNK細胞はIFN-γを産生する。IFN-γはマクロファージを活性化し，活性化マクロファージはIL-12やTNF-αを産生する。IL-12はNK細胞をさらに活性化する。一方，TNF-αは感染ウイルスの情報を取り込んだ樹状細胞の成熟化を誘導する。成熟化した樹状細胞はリンパ管に侵入可能となり所属リンパ節に到達し，ウイルス感染から細胞を守るための傷害性T細胞（CTL）や抗体産生B細胞が活性化，増殖するというシナリオである。

II. 腫瘍細胞は非自己か？

まず，厳密なシステムである獲得免疫系は，癌を非自己と認識しているのか？という問題である。10年ほど前に，癌細胞に特異性の高い種々の癌関連抗原（精巣特異的抗原であるMAGE蛋白など）のペプチドが癌細胞表面のMHC class I分子（ヒトではHLA class I分子）と結合し，CD8陽性T細胞に非自己として認識されることが主として*in vitro*の系で示された。最近では，CD4陽性T細胞が認識するMHC class II分子と結合した癌関連ペプチドも次々と報告されている。これらの多数の論文は，T細胞が癌細胞を非自己として認識し得ることを示している。一方，実際の癌患者由来の腫瘍細胞に対して明らかな傷害性を発揮するT細胞を患者末梢血やリンパ節あるいは腫瘍局所から手に入れることは甚だ困難である。また，実験的にT細胞が認識可能であることが証明されている抗原ペプチドを患者の抗原提示細胞上のMHC分子に乗せて，患者のT細胞が反応するかELISPOTアッセイなどにより検討してみてもほとんど反応は確認されない。つまり，患者は大きな癌を抱えているにもかかわらず癌細胞を非自己として認識するT細胞は患者の生体には極めて少数存在しているにすぎないと考えざるを得ない。いい換えると，「T細胞は癌細胞を非自己として認識する能力をもってはいるが，臨床の場で遭遇する癌細胞は患者のT細胞によってほとんど認識されていない」可能性があるということである。これには少なくとも二つの理由が想定される。一つは，臨床癌にまで発育した癌はすべて免疫系を逃れた癌だという考えであり，二つ目は，そもそ

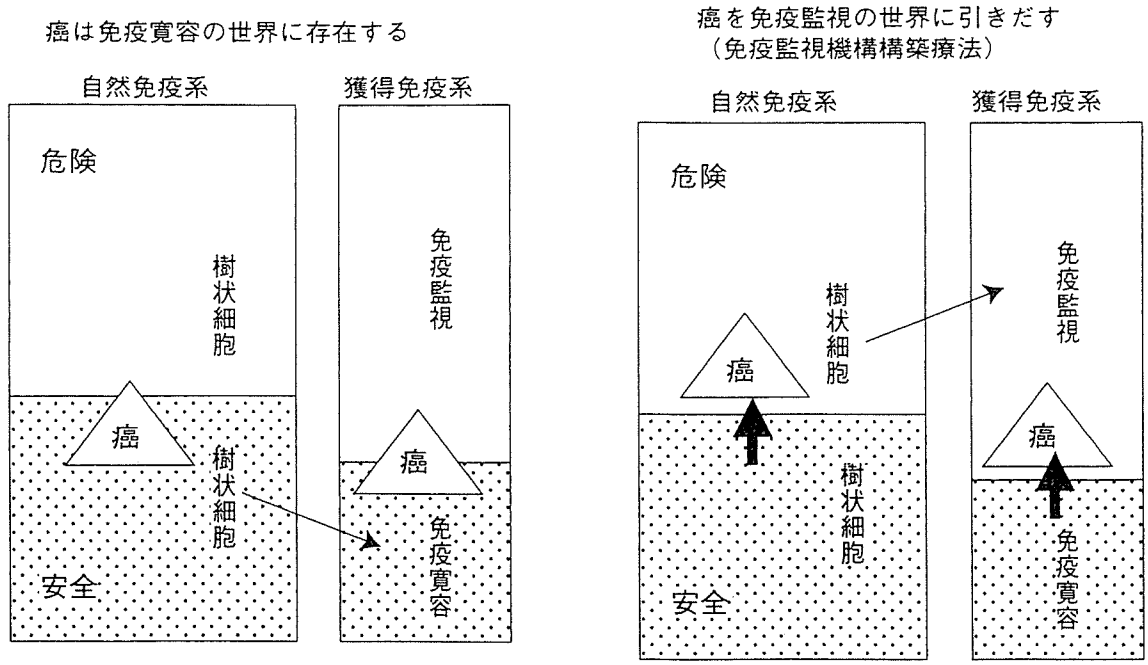

図1　腫瘍免疫の概要（仮説）
癌細胞は，獲得免疫系の世界からみれば非自己としての特性を有している。しかし，自然免疫系の世界では危険なものとは判断されない。癌が発生する組織において危険と判断されなければ，癌の情報は獲得免疫系へ伝達されない。つまり，癌に対する免疫監視機構は作動しない。もしも安全と判断されて癌の情報が獲得免疫系へ伝えられた場合は，癌に対する免疫寛容が誘導されてしまう。したがって，“免疫監視機構構築療法”の基本は癌細胞を自然免疫系に危険なものとして認識させ，さらに獲得免疫系における癌細胞の非自己性を高めることにある。そして大切なことは，生体をPSK投与や栄養改善などにより免疫系が作動しやすいように改善する努力である。

も癌は生体においては非自己というより自己として認識されているという考え方である。われわれは，後者すなわち，臨床癌は，「免疫監視機構の世界ではなく免疫寛容の世界に存在している」と考えている。

III.　腫瘍細胞は危険か？

前に述べたように，自然免疫系が危険と判断しなければ獲得免疫系すなわち免疫監視機構は動けない。では，癌局所において腫瘍細胞が危険であると自然免疫系が判断しているかという点である。特に，樹状細胞の活性化や成熟化を誘導するようなサイトカイン（IL-1，TNF-α，IFN-γ，IL-12など）が癌局所で産生されるかという問題である。なぜなら，未熟な樹状細胞は貪食による抗原捕捉力は高いが，リンパ管内への浸潤能，抗原処理能あるいはT細胞活性化に必要な補助分子の発現が弱い。われわれは，進行胃癌を対象に上記サイトカイン産生を検討したがmRNAレベルでも上記サイトカイン発現はわずか20%程度の症例で認められたにすぎなかった[1)]。一方，胃癌を含む他の癌局所では樹状細胞の成熟化を抑制するようなTGF-β，VEGFあるいはIL-10といったサイトカインの発現は約60%の症例で認められた[1-5)]。また，癌局所での癌細胞の死の形式はアポトーシスが主体と考えられるが，アポトーシス癌細胞を貪食した樹状細胞は壊死腫瘍細胞を貪食した場合に比べ，TNF-αやIL-12産生が低く成熟化の程度が弱いと予想される（Onishi, H.: 論文投稿中）。これらを総合すると，癌局所は感染局所とはまったく様相が異なり，樹状細胞が成熟化しにくい環境だと想像される。すなわち，自然免疫系は癌細胞を危険と認識していないことになる。何度も繰り返すが，自然免疫系が動かなければ獲得免疫系は動かない。

IV.　腫瘍免疫の概要（図1）

すなわち，腫瘍細胞は非自己としての特性は有

しており，T細胞は腫瘍細胞に反応する能力をもっている。しかし，腫瘍局所においては，自然免疫系が腫瘍細胞を危険なものと判断しないために腫瘍細胞の非自己としての特性（抗原性）は獲得免疫系へ伝わらない。むしろ，十分成熟化していない樹状細胞がリンパ節へ腫瘍細胞の情報を伝えている可能性があり，この場合には腫瘍細胞に対する免疫寛容（アネルギー）が誘導される。

V. 免疫監視機構構築療法

これまで，解説してきたように臨床癌は，「非自己としての特性を有してはいるが，免疫監視機構の世界ではなく免疫寛容の世界に存在している」と想像される。非自己としての特性を有するということは，癌細胞はワクチン療法の標的となり得るということになる。感染症に対するワクチン療法は非常に高い効果を上げている。したがって，腫瘍免疫の場を感染免疫の場のように変えれば効果的なワクチン療法が開発できると仮定した。われわれは，腫瘍免疫はそもそも免疫寛容の世界にあると予想しているので，再構築療法ではなく新たに感染免疫に似た免疫監視の場を作りだすという意味で構築療法と名付けた。

免疫監視機構構築療法の基本は，①生体を免疫反応誘導可能な状態に是正する。このために血清TGF-βレベルを低下させるなどの作用が期待されるPSK（クレスチン）を投与する。②腫瘍局所を炎症の場に変える。このために，少量のOK-432（ピシバニール）や活性化リンパ球を局所投与する場合がある。③自己腫瘍細胞で刺激した末梢血単球由来樹状細胞をワクチンとして使用する。原則としてOK-432あるいはOK-432刺激単核球の培養上清を用いて十分成熟化させた後，皮下投与する。④治療は2～3週に一度，可能な限り継続する。⑤治療中にCTL前駆細胞が増加してきた時点で末梢血リンパ球を採取し，OKT-3/IL-2で活性化させ，原則として静脈内投与する。

現在，次の臨床試験が進行中である。①癌性胸腹膜炎に対する末梢血幹細胞移植および樹状細胞（DC）ワクチン療法を基本とする免疫監視機構構築療法（2001年5月，九州大学医学部倫理委員会承認）②難治性固形腫瘍に対する免疫監視機構構築療法（2002年6月，九州大学医学部倫理委員会承認）③難治性固形腫瘍に対する他家腫瘍細胞による免疫監視機構構築療法（2002年6月，九州大学医学部倫理委員会承認）などである。

この治療と並行して実施された主な基礎研究の結果を紹介すると，①癌患者の単球由来樹状細胞には細胞寿命が短く抗原提示能の低下したポピュレーションが存在する[6]。②この細胞寿命の短いポピュレーションの割合とCTL前駆細胞誘導効率は逆相関を示す（Onishi, H.: 投稿準備中）。③OK-432はGMPグレードの樹状細胞の成熟化因子であり，特にIL-12p70産生誘導作用が高い[7]。④自己腫瘍細胞を用いることで，複数の腫瘍関連ペプチドに反応するCTL前駆細胞が末梢血中に誘導される。⑤画像や腫瘍マーカーでの追跡の結果からは，抗腫瘍効果を維持するためには治療の継続の必要性が示唆される。⑥感染症や肝機能障害は本療法の効果を低下させる。

現在まで，40例を超す症例に本療法を実施してきた。phase I studyおよびphase I / II studyの結果を解析中であり[8]（Morisaki, T.: 論文投稿中）詳細は報告できないが，有害事象としては軽度の発熱，一過性の好酸球増加（1例），一過性のIgE上昇（1例）などが認められた。本療法の適応基準を満たしながらも本療法を施行しなかった症例に比較し，本療法施行例は生存期間が有為に延長しており，生存期間の延長は入院期間の延長ではなく，外来通院期間の延長の結果であることが判明している（Nakamura, M.: 投稿準備中）。

VI. 腫瘍制御学分野で開発中のプロジェクト

1. 次世代免疫療法を想定した治療用腫瘍細胞バンクの開発

腫瘍免疫療法（特に癌ワクチン療法）の分野においては，標的となる腫瘍細胞は多くの未知の腫瘍抗原を含んでおり，極めて魅力的な治療材料でもある。しかし，現存する癌細胞株の多くは，異種血清（牛胎児血清など）で培養維持されており，治療用に用いるには未知のウイルス感染の可能性および使用目的に対するインフォームド・コンセントがなされていないなどの様々な問題がある。このため，治療に特化した質の高い安全な治療用腫瘍細胞バンクを計画実施中である。現在，このバンクに登録された治療用腫瘍細胞を用いて，

「難治性固形腫瘍に対する他家腫瘍細胞による免疫監視機構構築療法（2002年6月，九州大学医学部倫理委員会承認）」を実施中である。

2. 癌細胞由来エクソゾームによるワクチン療法の開発

われわれは，単球由来樹状細胞が分泌する微細小胞（直径30～100 nm）であるエクソゾームにMHC分子やCD86などの抗原提示に必要な分子が存在しており，CD4 T細胞を活性化する能力があることを確認した（Matsumoto，K.：論文投稿中）。さらに，癌細胞もエクソゾームを分泌し，癌細胞由来のエクソゾームには抗原ペプチドのシャペロンとして働くheat shock proteinなどの発現が確認されている。現在，これら樹状細胞由来および癌細胞由来エキソゾームを応用した新たなワクチン療法の可能性を検討中である。

3. 抗体療法と細胞療法の併用療法

HER2強陽性再発乳癌に対してHerceptin療法が保険適応され，一定の臨床効果を上げつつある。われわれは，*in vitro*の系でHerceptinがNK細胞などを介して抗体依存性細胞介在性細胞傷害作用（ADCC）を発揮することを確認した[9]。さらに興味あることには，Herceptinによる直接的な増殖抑制が認められないHER2低発現乳癌細胞に対してもADCC効果は発揮された。また，OK-432やIL-2などで活性化したリンパ球を用いた場合にも同様のADCC活性が認められる。癌組織は免疫学的に不均一な集団であり，HER2強陽性乳癌といえどもHER2発現の低い癌細胞は存在する。したがって，Herceptin療法に細胞療法を併用すれば，これらHER2発現の低い癌細胞に対する細胞傷害が発揮され，有効な治療法となることが期待される。さらに，Herceptin療法の対象をHER2過剰発現乳癌からHER2陽性乳癌に広げることも可能になるかもしれない。現在，「HER2/neu過剰発現転移性乳癌に対するHerceptin/免疫細胞療法併用療法（2003年2月，九州大学医学部倫理委員会承認）」を実施中である。

また，乳癌での臨床効果が確認されれば，HER2発現が高い膵癌あるいは胃癌へと拡大し得る可能性もある。

4. 抗癌剤（サイトカイン）および抗アポトーシス経路遮断剤併用療法

癌組織中の癌細胞は，低酸素状態，発熱，様々なサイトカイン，活性酸素など，実に多彩なストレス環境のなかで活発な増殖能を維持している。すなわち，これらストレスから自身を保護する機序（抗アポトーシス活性化経路）を有していると思われる。われわれは，「抗癌剤やサイトカインは癌細胞にアポトーシス経路の活性化と同時に抗アポトーシス経路も活性化する。したがって，抗癌剤やサイトカインの量を増やすのではなく，抗アポトーシス経路を遮断すればより低濃度でも抗腫瘍活性は増強する」という作業仮説を立て，その解析を進めてきた。われわれは，大腸癌や胃癌組織中の癌細胞において代表的な抗アポトーシス経路を形成するnuclear factor kappa B（NF-κB）が持続的に活性化されていることを見いだした[10-12]。したがって，抗癌剤やサイトカインが果たして癌細胞のNF-κBを活性化させるかを検索したところ，taxane系薬剤であるdocetaxelやサイトカインであるIFN-γが種々の癌細胞に対してアポトーシス経路と同時にNF-κB活性化を誘導することがわかった。また，これら薬剤によるNF-κB活性化を，移植医療において免疫抑制剤として臨床使用されているcyclosporin A（CsA）やFK506あるいは経口免疫賦活剤であるクレスチン（PSK）が抑制することを見いだした[13-17]。これらの基礎データを基に，CsAやFK506を投与中の移植患者の癌治療におけるIFN-γあるいはIFN-α（内因性IFN-γの誘導作用が報告されている）治療，あるいは難治性の胃癌や膵癌に対するtaxane/PSK療法およびtaxane/CsA療法の開発をめざしている。

これら薬剤によるNF-κB活性化は，単に癌のアポトーシス抵抗性に関与するだけでなく，MMP 9の活性化などを通して癌細胞の浸潤にも関与している[18]。したがって，PSKやCSAはNF-κB活性化抑制作用を通して癌の浸潤，ひいては転移を抑制することも期待され，NF-κB経路を標的とする薬剤として応用し得る可能性がある。

5. シグナルクロストークを利用した腫瘍制御

われわれは，胃癌の術前悪性度評価に基づく遺伝子カルテの作成を目標として，癌細胞の新生血管形成，浸潤や転移あるいは免疫抑制に関与する

種々の因子の胃癌組織での発現をmRNAレベルで検索してきた[4,19]。その結果，PDGF-AとTGF-βのmRNA発現程度と生存期間が逆相関を示すことや，TGF-βが浸潤能と強い相関があることなどを見いだした[20,21]。この臨床研究のデータに基づき，「TGF-βのシグナル系を直接ブロックするのではなく，癌細胞がすでにもっているTGF-βによる浸潤に関与するシグナル系とクロストークする別のシグナル系を利用することで，より生理的に浸潤を制御し得る」と仮定し，TGF-β依存性浸潤を示す胃癌の三次元培養モデルを作製し，このモデルを用いて胃癌細胞における細胞内のシグナル伝達系について解析した。その結果，TGF-βは胃癌細胞のSmad 2/3のリン酸化を引き起こし，リン酸化Smad 2/3-Smad 4複合体が核内へ移行し，MMP 9やuPAの転写が亢進し，結果として浸潤能が亢進すること，さらにはIFN-γがJak/STAT系を介してSmad 7の発現を増強させ，増加したSmad 7がTGF-βによるリン酸化Smad 2/3-Smad 4複合体の核内移行を抑制することが明らかになった[15,22]。すなわち，TGF-βによるSmadシグナル系の活性化を，IFN-γはJak/STAT系を介して抑制する可能性が示されたわけである。現在，TGF-β依存性の浸潤癌に対し，増殖抑制ではなく浸潤能抑制という「特定の悪性病態を標的としたIFN-γ療法」の可能性を解析中である。したがって，われわれはこの現象を，「シグナル系のクロストークを利用した腫瘍制御法」と名付けている。このような方法を利用することで，従来の分子標的治療とは若干異なったより生理的な治療法が開発可能だと期待している。

6. 癌局所の炎症反応制御による悪性度制御

炎症と癌の悪性度との関連性は古くから指摘されてきたが，分子レベルでの解析結果の報告は少ない。上述したように，胃癌組織においてはNF-κBが活性化しており，このNF-κB活性化と癌細胞の浸潤能には統計学的な相関が認められた[12]。一方，IL-1βをはじめとする炎症性サイトカインはマクロファージのNF-κBを活性化させる。われわれは10年以上前から，「癌とは血球様形質が再び蘇った幹細胞に近い細胞である」と考えて研究してきた。したがって，「IL-1βは癌細胞のNF-κB活性化を通して癌の浸潤能を高める」という仮説を立てて研究をスタートした。その結果，IL-1βが胃癌細胞のNF-κBを活性化し，NF-κB活性化がMMP 9の発現増強を誘導し，結果として癌細胞の浸潤能を亢進させるという仕組みが存在する可能性が明らかになった[18]。すなわち，炎症が癌細胞の悪性度を増強する可能性が分子レベルで示唆された。現在，IL-1β以外の炎症関連物質が癌細胞の種々の性質にどのような影響を及ぼすのか，果たして*in vivo*においてもこのような現象はみられるのかを動物モデルおよび臨床検体を用いて解析している。また，同時に治療法開発を目的として，癌局所での炎症の制御が果たして癌の悪性度を制御し得る可能性があるかの検討に入っている。炎症は免疫反応と強い相関があり，治療法として確立するには極めて複雑なシナリオを描く必要があるが，炎症という生体反応と癌の悪性度の関連を明らかにしていく作業は新たな治療法開発へと導いてくれると信じている。

7. 新たな視点に立った免疫化学療法

化学療法に免疫療法を組み合わせる方法は古くから行われてきた。この理由の一つは，免疫療法による上乗せ効果を当然期待したものだが，われわれは以前より，「化学療法剤のような副作用を主作用と期待して使用する薬剤の場合には，免疫療法を抗腫瘍効果の上乗せという視点とは別に，化学療法剤使用前に生体の免疫能をできるだけ整えるという視点からも実施するべきである」ことを訴えてきた。この考えに変わりはないが，これまで紹介してきたようにPSKは免疫賦活剤としての作用とは別に，NF-κB活性化抑制作用を通して癌細胞の浸潤能などに影響を与える場合がある[16,18]。さらに，種々の抗癌剤が癌細胞にNF-κB活性化を誘導し，癌の悪性化を増強させる可能性も明らかになってきた[16,17]。すなわち，PSKは癌細胞に対する浸潤能抑制剤，あるいは抗癌剤による悪性化の防止薬として作用する可能性がある。さらに，PSKが癌細胞のTGF-β産生を抑制し，癌の浸潤を抑制する可能性もある[14]。われわれが10年ほど前に報告した「大腸癌切除例に対するPSKの再発予防効果」は，この可能性を裏付けるデータかもしれない[23]。このような視点に立った新たな免疫化学療法の開発も可能であろう。

さらに，taxane系薬剤がNK細胞を中心とする免疫担当細胞の細胞傷害活性を増強することとその機序の一端が明らかとなった（Kubo, M.: 論文投稿中）。これらを総合すると，taxane系薬剤にPSKを併用した場合，PSKは癌細胞のTGF-β産生を抑制し免疫能を改善し，taxaneはNK細胞の傷害活性を高める。一方，癌細胞に対してはPSKは癌細胞のNF-κB活性化を抑制し，taxaneによる癌細胞のアポトーシス誘導を増強する。さらには，taxaneによるNF-κB活性化を通して増強された癌細胞の浸潤能をPSKは抑制するというシナリオも想定することができる。現在，難治性の固形癌を対象としたtaxane/PSK併用療法の実施作業に入っている。

おわりに

われわれは，「癌細胞を自然免疫系が危険と判断しないために，癌細胞は免疫寛容の世界に置かれている」と考えている。したがって，癌局所において自然免疫系が危険だと判断するように，癌局所を疑似感染局所に変えた上で，さらに獲得免疫誘導効率を高めるために樹状細胞ワクチン療法と活性リンパ球移入療法を併用する“免疫監視機構構築療法”といった複雑な治療法を実施している。この療法そのものは，複雑すぎて治療法としての直接的貢献は少ないかもしれない。しかし，できるだけ基礎研究のエビデンスに沿った丹念な治療を工夫することこそがより容易な治療法開発の王道であると信じている。また，免疫監視機構構築療法と並行して進行中の開発型医療研究のプロジェクトの一部を紹介した。紙面の関係上，具体的な研究成果の詳細なデータ記載は省略した。その結果，非常に観念的で雑駁な内容となったことを深く詫びなければならないが，免疫学を基盤とした新たな癌治療法を開発するための参考にしていただければ幸いである。

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