Histological and Ultrastructural Findings of Regressing Canine Transmissible Venereal Tumor After Repeated Transplantation

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Abstract. The structure and behavior of canine transmissible venereal tumor after repeated transplantation were studied. Ten random mongrel dogs were divided into a control group (Nos. 1–5) and an experimental group (Nos. 6–10). Before the investigation the experimental animals were sensitized repeatedly by subcutaneous injections of a tumor cell suspension. The cell suspension (cell viability=22.0–38.8%) was transplanted subcutaneously into the right and left hypogastric regions, and within 4 to 5 days after transplantation, a palpable tumor with nodules of 3–5 mm in diameter was detected. By the 18th day after transplantation, the tumors of the experimental animals were not observable. The regression of the tumor in the experimental animals showed the following pattern: firstly, the tumor cells were infiltrated by lymphocytes; secondly, the lymphocytes adhered to the surface of the tumor cells; thirdly, the tumor cells were destroyed and ultimately reabsorbed. Electron microscopy of the tumor cell destruction revealed the following: some of the lymphocytes adhered to the tumor cell at the surface of the cell membrane running parallel to it; when the lymphocytes came into contact with the tumor cell, an intedigestion of protuberances of both cells was visible; the surface of the tumor cell membrane was disturbed at the point of contact; the tumor cell appeared swollen and showed an irregular form; and the cytoplasm was vacuolated with many electron-dense granules. As above mentioned, lymphocytes are thought to have an important role in the regression of the tumor.

Canine transmissible venereal tumor has a well-known and interesting feature that the apparent immunity is followed by spontaneous regression or surgical removal of the tumor [3, 20]. Canine transmissible venereal tumor usually develops on the external genitalia of both sexes and is naturally transmissible from dog to dog through copulation. Its incidence is worldwide. It is also transplantable experimentally. The first known transplantable neoplasm was transplanted subcutaneously from one dog to another by Novinsky as early as 1867 [23]. The tumor is transmitted at coitus by viable cells, and the same line of cells passes yearly from dog to dog. Although karyotypic differences exist between normal dog cells and the tumor cells, it has been demonstrated that the tumor samples from experimentally transplanted and primary neoplasms have strikingly similar karyotypes [17]. In our laboratory, the tumor has been passed through 50 generations of puppies since 1967 by subcutaneous injections [24, 15].

It is said that cancer immunity is mediated by lymphoid cells. In the studies of the direct effect of lymphoid cells on target cells in vitro, many investigators have pointed out that the lymphoid cells adhere to and aggregate around the target cells and the tumor cells are destroyed [7, 8, 12, 13,
18, 19, 22].

Our purpose in this study was to examine the histological and ultrastructural findings of regressing canine transmissible venereal tumor after repeated sensitization by subcutaneous injections of the tumor cell suspension.

**Materials and Methods**

Animals and Transplantation: Ten random mongrel dogs aged 2 and 9 months were divided into control (Nos. 1–5) and experimental animals were sensitized repeatedly by subcutaneous injection of the tumor cell suspension [14, 15]; the details of inoculation are given in Table 1. The duration was measured from the first appearance of the tumor until it was no longer present as determined by palpation. No specimens were obtained from donors of tumors which had been used in previous transplantations. The removed tumor was minced to a brei with scissors, filtered once through gauze, and then suspended in Ringer’s solution containing 2000 units/ml of penicillin. Viable tumor cell counts of the cell suspension were checked by the eosin dye exclusion test [16]. The tumor cell suspension (cell viability=22.0–37.8%) was transplanted subcutaneously into the right and left hypogastic regions of the control and experimental animals.

Preparation of Specimens: The samples which were removed on the 4th, 7th, 10th and 18th days after transplantation were processed for light and electron microscopy. For light microscopy, the samples were immediately fixed in 10% buffered formalin, conventionally treated for preparation of paraffin sections, and stained with hematoxylin and eosin. For electron microscopy, the specimens were fixed in 2% glutaraldehyde solution for 2 hours and 1% osmium tetroxide in ice. The tissue was then rewarshed and embedded in Epon 812. Sectioning was done with LKB ultratome using glass knives. Acceptable specimens were stained with uranyl acetate and lead citrate, and observed with a H-12A electron microscope.

**Results**

General and Light Microscopical Observation: In most of the experimental and control animals palpable growth was observable in the sites of implantation at approximately the same time. The first tumor, with nodules of about 3–5 mm in diameter, appeared at 4 and 5 days after injection of the tumor cell suspension. Examination of the removed material revealed the generally wellknown pattern of canine transmissible venereal tumor, i.e. a neoplasm made up of round or polyhedral closely packed cells with a little cytoplasm, and a round nucleus with scattered chromatin and a prominent nucleolus (Fig. 1). Mitotic figures were usually seen.

There was no difference between the experimental and control groups until the 7th day after which the tumors of the control group increased greatly in size while those of the experimental group were gradually reabsorbed. On the 10th day after injection, the experimental group displayed many lymphocytes among the tumor cells (Fig. 2), although the lymphocytes were not connected to the tumor cells. On the 14th day after injection, the tumor cells of the experimental group decreased markedly in number and the lymphocytes became attached to the tumor cell (Fig. 3). On the 18th day, there were no tumor cells observed, only the lymphocytes (Fig. 4).

Electromicroscopic Findings: Generally, the healthy tumor cells (Fig. 5) had a round or oval nucleus with a nuclear membrane which was frequently pocketed with cytoplasmic extensions. The nucleoplasm was homogenous and delicate except where the
chromatin condensed along the nuclear membrane. A characteristic large nucleolus was present. In the cytoplasm, the ribosomes were free or in the form of polysomes, and they were irregularly distributed. A few strands of granular endoplasmic reticulum were seen. Various grouping of round or oval mitochondria containing a few cristae were present. The Golgi apparatus was small. Electron-dense granules and vacuolations of the cytoplasm were occasionally seen. The cell surface showed a characteristic arrangement of microvilli.

On the 10th day, The lymphocytes attached to the tumor cells were usually uniform in size and close to the external membrane of the tumor cell over a considerable area of the cell surface. The tumor cell (Fig. 6) appeared to be in the early stages of degeneration and had many cytoplasmic vacuoles, several electron-dense bodies, and a wide perinuclear space. As exhibited in Figs. 6, 7, 8, the adjacent plasma membranes at these points of approximation remained nearly parallel; the lymphocytes were in contact with the tumor cell, revealing interdigitation of the protuberances of both cells; and the surface of the tumor cell membrane was disturbed at the point of contact. There was little nuclear chromatin except where it had condensed along the nuclear membrane. The mitochondria appeared somewhat swollen and had only a few thin cristae. Polysomes were scattered through the cytoplasm. Electron-dense and membrane-bound bodies were apparent. On the 14th day, the tumor cell appeared swollen and the cell membrane was obscure. The nuclear chromatin was condensed, and irregular in form. The vacuolated cytoplasm revealed many electron-dense and membrane-bound granules with a degenerating tumor cell which had formed various bulbous blebs of cytoplasm.

Some of the blebs were seen in the outer tumor cell. The blebs contained dispersed ribosomes, an endoplasmic reticulum, and some mitochondria (Figs. 9, 10, 11). On the 18th day, there were no tumor cells, and only several lymphocytes observed. Dilated profiles of the granular endoplasmic reticulum containing an amorphous material, electron-dense and membrane-bound bodies, undifferentiated bodies, and a lysosome-like structure were seen (Fig. 12).

**Discussion**

The histological and ultrastructural characteristics of the tumor cells observed in this study were similar to those previously reported [1, 2, 4]. We studied the regression of these tumor cells histologically in experimental animals which had been sensitized repeatedly by subcutaneous injections with the tumor cell suspension. The following pattern emerged: firstly, lymphocytes adhered to the surface of the tumor cells; thirdly, the tumor cells were destroyed and ultimately reabsorbed. Although the initial stages leading to lysis, e.g., the linking of the effector cells to the tumor cells, are still not clearly understood, the terminal events have already been quite well characterized. Based on findings from several laboratories, Henney [10, 11] classified the T-cell induced lytic cycle into five stages: specific binding, stimulation, membrane permeability change, osmotic swelling, and lysis.

In the present study, according to the histological changes of tumor cells, the following four stages of lytic cycle were distinguished. 1) Specific binding: many lymphocytes adhered to the surface of the tumor cell (Fig. 6). (2) Membrane permeability changes: The tumor cell membrane was obscure (Fig. 8). (3) Osmotic swelling: the cytoplasm appeared swollen, and the nuclear form was irregular (Fig. 9). Lysis:
the tumor cell showed a degeneration which was characterized by various bulbous blebs of cytoplasm, some of which were present in the outer tumor cell (Figs. 10, 11).

In this canine transmissible venereal tumor, the increasing numbers of degenerating tumor cells in the regressing tumors, coupled with the association of increasing numbers of lymphoid cells, indicated an immune response to the tumor cells [3, 20]. The possible involvement of the immune system in the destruction of the tumor cells has been a matter of concern for several decades. The first affirmative answer resulted from Govaert's [5] demonstration of the direct lytic activity of lymphoid cells in vitro. In a quantitative study of the immunologic activities of sensitized lymphoid cells on homologous tumor cells, Winn [24] suggested that lymphoid cells act directly on the tumor and that a close association between the lymphoid cell and the tumor cell is necessary for tumor growth inhibition. Mannami [20] observed that when the lymph node cells sensitized by the Ehrlich ascites tumor were mixed and cultured with JTC-11 cells derived from the same tumor, the interaction of the two cell groups exhibited a contactual phenomenon accompanied by destruction of the JTC-11 cells. In the interaction where the lymph node cells become attached to the JTC-11 cells resulting in the destruction of JTC-11 cells, the lymph node cells also destroyed. In our observation, however, the lymphocytes were not destroyed.

Other writer have reported that the rupture of the plasma membrane of tumor cells following interaction with the complement system or with "immune" cells occurs by colloid osmotic lysis [7]. Moreover, the interaction leads to the occurrence of a lesion in the target cell membrane through which enters the target cell. The target cell then swells until the cell membrane is explosively ruptured [21]. Figs. 10, 11 in our study suggest the mechanism of colloid osmotic lysis. The above-mentioned observations have been shown to hold in such diverse systems as the complement-induced lysis of Ehrlich ascites tumor cells and in the T-cell-mediated lysis of mouse mastocytoma cells, and they have led to the assumption that the terminal stages in tumor cell destruction by the immune system are identical [6, 7].

Kinetic evidence shows that target cell destruction results from collision with an effector lymphocyte [21]. Recent findings suggest that the initial lytic lesion on the target cell membrane appears during the period of contact between the effector T lymphocyte and its homologous target cell [9]. Figs. 6, 7, 8 suggest that a correlation exists between the lymphocyte and the tumor cell membrane. Figs. 9, 10, 11 show that after initial adhesion, the lymphocyte is unnecessary, and that the demise of the tumor cell resulted from its own inability to handle the augmented influx of water into its cytoplasm [10].

It is reasonable to presume that our observation fits the description of cell-mediated immunity as "the direct lytic action of immune thymus-derived (T) lymphocytes" [11]. It may be concluded that the effector cell in a specific response to the transplanted tumor is a lymphocyte in vivo.

References


要約
反復接種された大の可移植性細胞腫瘍における未熟像の光学的および電顕的所見について：大友亜十郎・小池幸男・工藤明一・酒井 保（北海道大学医学部家畜外科学教室）——我々は1967年以来大の可移植性細胞腫瘍を雌メスラットに代謝している。今回の、この腫瘍を使用して反復接種した場合の培養細胞を組織学的に検討した。10頭のラットを2群に分け、実験群は1群に先だつ腫瘍細胞を連続培養し、他群は接種した。実験群と対照群の両群の下腹部に腫瘍細胞浮遊液（生残率22.0〜38.8）を接種した。両群ともに接種後、4〜5日目および3〜5mm大の腫瘍を触知し得た。以後対照群の腫瘍は次第に増大するのに対し、実験群は10日目以降次第に消退し18日目には触知不能となった。次にそれぞれの組織所見を検査した。実験群では、腫瘍細胞間にリンバ球が浸潤し、リンバ球が腫瘍細胞の周囲に密着し、続いて腫瘍細胞が破壊され、最後に吸収された。さらに腫瘍細胞の変態細胞を観察すると、最初に腫瘍細胞とリンバ球の細胞膜が互いに平行に密着し、次いで膜面が相葉となり、変態細胞が互いに入り込んでいるように見える。腫瘍細胞内には大小不等の種々の形のdense bodyが多数出現した。また小胞体は全般に拡張し、空胞状を呈し、また核形は不定形となった。ことで細胞の構造が不明瞭となった。細胞は全体的に腫大し、ribosome様粒子を含む包状物が多数観察された。最後に腫瘍細胞の破壊細胞中に多数のリンバ球が観察された。以上の所見より、腫瘍細胞の破壊にはリンバ球が重要な役割を担っているものと考えられる。

Explanation of Figures
Figures 1 through 4 are light micrographs, and Figures 5 through 12 electron micrographs.

Fig. 1. The tumor of control animals composed of round or polyhedral, closely packed cells, with scarce cytoplasm and a round nucleus having scattered chromatin and a prominent nucleolus. ×800.

Fig. 2. On the 10th day after transplantation. The tumor is infiltrated by the lymphocytes. ×800.

Fig. 3. On the 14th day, the lymphocytes adhere to the surface of the tumor cell, and the change of the tumor cell is observed (left lower). ×800.

Fig. 4. On the 18th day, the tumor cells are destroyed, and the large vacuole is surrounded by many lymphocytes (center). ×800.

Fig. 5. The tumor cells of control animals, showing a large nucleus which contains a nucleolus, and cytoplasmic projections or microvilli. ×6,000.

Fig. 6. On the 10th day, the lymphocytes are in contact with the tumor cell. Cytoplasmic vacuoles and electron dense bodies are observed in the tumor cell. ×17,000.

Fig. 7. A interdigitations of protuberances of both cells (lower) is visible. ×29,000.

Fig. 8. The surface of the tumor cell (TU) membrane is disturbed at the point of contact with the lymphocytes (LY). ×40,000.

Fig. 9. On the 14th day, the tumor cell is surrounded by several lymphocytes and shows irregular nuclear form. ×7,000.

Figs. 10, 11. The tumor cells appear swollen with an irregular nucleus. ×7,000, ×5,000.

Fig. 12. On the 18th day, only several lymphocytes are observed, but no tumor cells. ×6,000.