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T-LYMPHOCYTE MEDIATED TUMOR CELL DESTRUCTION *IN VIVO* ASSOCIATING WITH A SPECIFIC FEATURE OF APOPTOSIS

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The immunological responsiveness of the T-cell immunodificient NC nude mice tumor models was reconstructed by thymic transplantation of the semi-dominance NC mice. After immune reconstruction (IR) the tumor continued to grow until 2 to 3 weeks, then the volume of the tumor reduced gradually and disappeared at the 9th week. In both H901 and SW1116 solid tumor nodules was found by light microscopic study (IM), after IR, tumor cells gradually replaced by lymphocytes and fibroblasts, shrinked till only isolated cell groups, then totally disappeared. The whole processes like that the tumor cells were nibbled. It was found that the main tumor cell death related with a specific feature of apoptosis, which had typical dense chromatin distributed along the inner surface of the nuclear membrane by transmission electron microscopic study (TEM). The IR model could be useful for further mechanical research of immune system.

Key words: Nude mice, Human colon cancer, Immune reconstruction, T-lymphocyte, Apoptosis.

Apoptosis,[1,2] a special form of cell death, has become a major focus for tumor cell death.[3-5] Recently, it has been clear that tumor cell death is a far more complicated process than one might anticipate. We have established a model which could destroy human cancer during the process of the immune reconstruction (IR) in T-cell immunodeficient nude mice. The model provides us a point of view to observe the immunological effect of T-lymphocyte on human tumor cells. This work was performed with morphological changes that accompanied the tumor cell death *in vivo*, which was mediated by T-lymphocyte using special IR model. We have focused our study on the process of the tumor destruction, which would undoubtedly be of great value for further understanding of the basic mechanism of the anti-tumor effects by immune system.

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MATERIALS AND METHODS

Animals

NC nude mice were obtained from Neurology Research Center, Su Zhou Medical University (a Japanese mouse strain). The nude mice were bred and cared in special pathogen free (SPF) conditions. Animals were used at age of 6 to 8 weeks and caged in groups of 6 or less.

Tumor Models

Two tumor models were involved in this study. The one was HCANZ-901 model (briefly H901 model), which had been xenografted subcutaneously with 1.5 mm^3 fresh surgical specimen of human colon cancer into NC nude mice, and the tumor maintained

in NC nude mice for 36 passages was used. The other was SW1116 model, which had been induced with 1×10^7 cells of SW1116 colon cancer cell line in NC nude mice subcutaneously first, and the tumor was maintained for 5 passages in NC nude mice before our experiment.

The Models of Immune Reconstruction and Tumor Destruction (IR-Model)

After the tumors of the model had grown 3 weeks, the thymus of same clan from 3 week old semi-dominance NC mice was planted subcutaneously into the tumor-bearing model mice, which was named the IR-model.

Distributions of Animals

Sixteen NC nude mice were used to each of two tumor models. They were divided into 4 control mice (no IR groups) and twelve experimental mice (IR groups). Six mice in IR groups were sacrificed in order on weeks 0, 1, 2, 3, 4, 5 for morphological studies, and the other 6 mice were observed till all tumors disappeared.

Morphological Studies

The morphological characteristics of the two tumor IR-models were observed, including light microscopic study (IM) and transmission electron microscopic study (TEM).

Experimental Procedures

Xenografts were established by subcutaneous implantation of the human tumor fragments into the lateral costal area of NC nude mice. When the local tumor reached 7–8 mm in diameter just within 21 days, the immune reconstruction was then in progress. For assessment of the T-lymphocyte mediated human tumor cell destruction, we monitored tumor growth or inhibition by measuring the tumor diameter with a caliper and calculated the tumor volume (V) by the following equation: $V= a\times b^2/2$, where a is the length of the tumor in millimeters (large diameter) and b is the width of the tumor (small diameter). Within the duration, light microscopic and ultrastructure studies were undertaken every week till fifth week according the finding of the pretest. The endpoint of the experiment was that all remnants of tumors of IR-models were absorbed. Then the tumor inhibitive curves were drawn.

RESULTS

Phenomenal Study

The tumor of the no IR mice bearing with both of H901 and SW1116 had the tendencies to unrestricted growth; however the tumors of the IR mice bearing with the both, after the continued growth in the following two or three weeks, the volume of the tumor reduced gradually till they had disappeared at 9th week of IR (Figure 1). After 4 weeks, the transplanted thymuses of the semi-dominance NC mice were disappeared from the subcutaneous region.

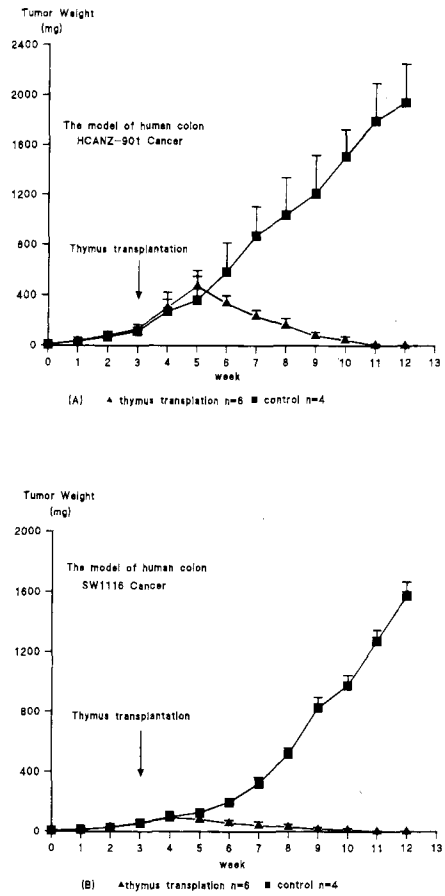
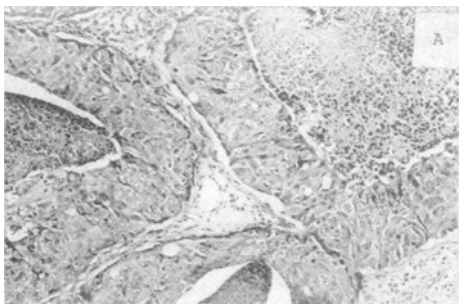


Fig. 1. The process of tumor destructions after IR in both H910 (A) and SW1116 (B) bearing NC nude mice.

Morphological Study

The light microscopic study (IM) had shown there was no obvious histralogical changes in one week (Figure 2, 3). In the following three weeks, the tumor cells were destroyed gradually (Figure 4–6). In fifth week the morphological feature were characterized by hazy wreckage of tumor cells, prominent lymphocytic infiltration and fibroblastic prolifera-



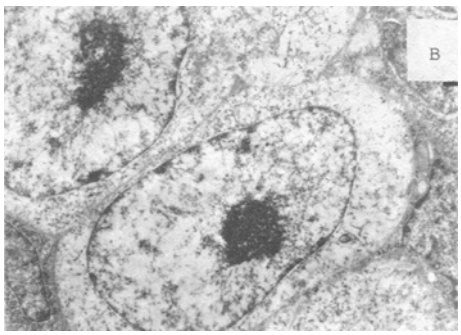


Fig. 2. The human colon adenocarcinoma H901 grows in NC nude mice.

A. light microscopic study (IM). × 200

B. transmission electron microscopic study (TEM). × 7100

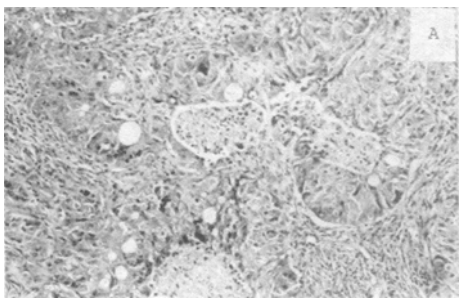
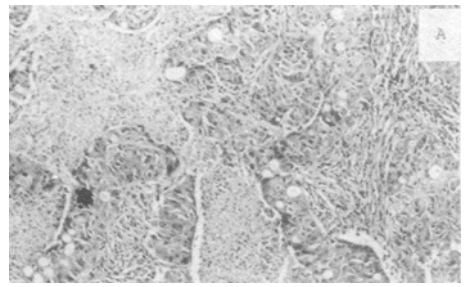


Fig. 3. One week after IR.

A. the morphological featurees of H901 was no obvious changes, except a little increasing of lymphocyte in the connective tissue. IM × 200

B. transmission electron microscopic study. TEM × 7100



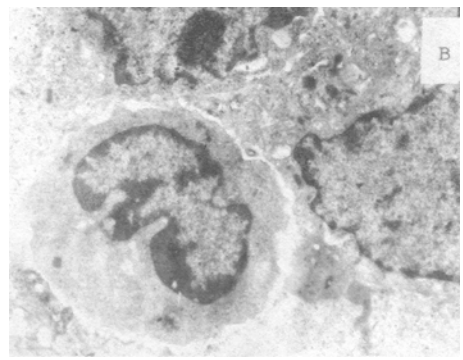
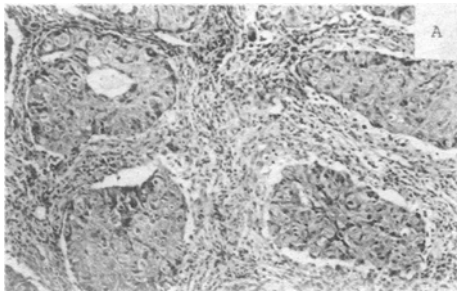


Fig. 4. Two weeks after IR

A. H901 were partially destroyed. IM × 200

B. the nuclei of apoptotic cells, dense chromatin along the inner surface of the nuclear membrane TEM × 4400

tion (Figure 7). At 9th week all remnants of tumors were absorbed. The transmission electron microscopic study (TEM) had shown clear apoptosis from the beginning at the end of 2nd week (Figure 4–6). The nuclei of the apoptotic cells had dense chromatin along the inner surface of the nuclear membrane, and the structure of the cells were also disappeared later (Figure 5, 6).



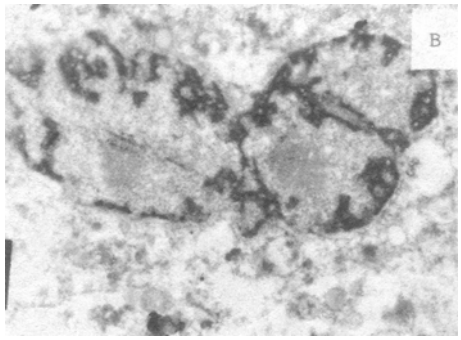
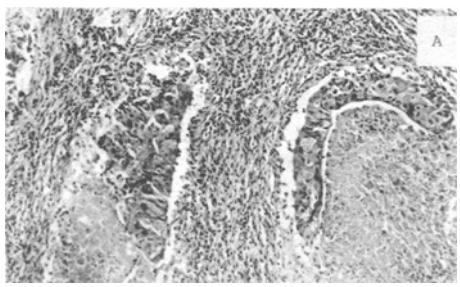


Fig. 5. Three weeks after IR.

A. H901 were markedly destroyed, surrounded by the connective tissue with lymphocyte infiltration. IM × 200

B. nuclei of apoptotic cells, dense chromatin along the inner surface of the nuclear membrane, the structure of the cells were disappeared. TEM × 4400



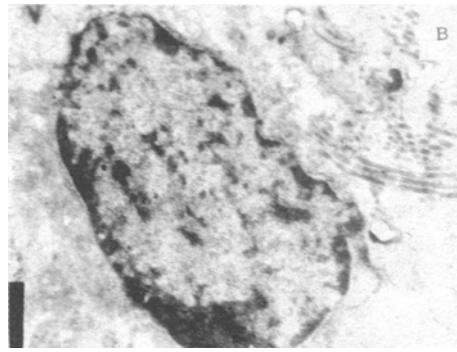


Fig. 6. Four weeks after IR.

A. H901 were severely destroyed, tumor shown as fewer isolated cell groups. IM × 200

B. nuclei of apoptotic cells, dense chromatin along the inner surface of the nuclear membrane, the structure of the cell were disappeared. TEM × 10400

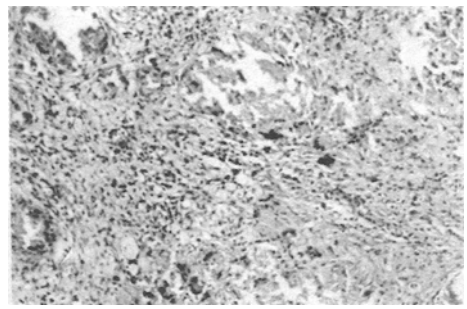


Fig. 7. Five weeks after IR only hazy wreckage of tumor cells were shown, replaced with prominent lymphocyte infiltration and fibroblastic proliferation. IM × 200

DISCUSSION

In this study, two kinds of human colon cancers were destroyed, after the T-lymphocyte adoptively transferred into the T-cell immunodeficient NC nude mice by thymic transplantation of the semi-dominance NC mice. The special finding in IM study was that in both H901 and SW1116 solid tumor nodules, after IR, tumor cells gradually narrow the range, shrinked till only isolated cell groups, then totally disappeared (the Figures of SW1116 IR model not shown here).

The whole processes like that the tumor cells were nibbled. This is a different mode comparing

with the spontaneous tumor necrosis, which was present in the centers of almost all tumor nodules, but not in the vigorous proliferative regions. TEM study was found that ultrastructurally tumor cell death in both H901 and SW1116 models, after IR, has the same characteristic, i.e. the aggregation of nuclear chromatin, and the dense chromatin distributed along the inner surface of the nuclear membrane. And then the cell membrane was broken-down and the structure lost, the major morphological features just as described by Kerr, et al..[1, 2]

According to the literatures[1,2,5] APO is characterized by chromatin condensation, apoptotic bodies, and DNA fragmentation. The feature could be detected some hours or few days after treatment. Although apoptotic bodies and DNA fragmentation are important parameters, they could not be always observed[6–8] or caught.[9] In this experiment we would like to investigate how APO occurs *in vivo* in solid tumor. In this approach apoptotic bodies or DNA fragmentation (data not shown) were not found at any stages, but the special chromatin condensation was detected. Seeking the reason, in this special model the total tumor cell death induced by T-lymphocyte and immune system had a more than one months' duration, so it was differentiate from previous reports.

About the rarer of the APO bodies, the hypothesis of the probable reason may be their engulfment by adjoining cells is usually rapid, and may even begin before they have completely separated from the condensing parent cell.[2] In this work, since the occurrence of APO was much longer than the other reported data, so there is little doubt if there is an another hypothesis that the tumor cell membrane as well as the components might be little bitter severely damaged in tumor tissue than in the other conditions, then resulting slight changes of the process of the second stage of APO and few finding of the APO bodies as well as DNA fragmentation. So the assessment of APO, especially *in vivo*, is worth to be further investigated.

Many different factors can induce tumor cell death. Although APO could be observed rarely in spontaneous tumor death,[1, 3] the nature process of the tumor growth is without restriction. Our result suggested that the T-lymphocyte mediated tumor destruction is related to the APO, and caused totally tumor cell death, the real mechanism is very complex.

Accumulated data indicated that nude mice were not necessarily more susceptible to spontaneous and various carcinogen-induced tumors then heterozygote mice.[10] So, it is a special event that nude mice can bear human tumors. In this experiment IR model does give us a chance to observed the immunological surveillance mechanisms against tumors which has previously been attributed to T-lymphocytes. It is obvious that work has still to be done to determine the occurrence and its relative factors in the malignant neoplasms. Our work suggested the IR model could be useful for further mechanical research of the immune system.

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