Effect of IgG Produced by Tumor-infiltrating B Lymphocytes on Lung Tumor Growth

MAKIKO MIZUKAMI, TAKESHI HANAGIRI, YOSHIKI SHIGEMATSU, TETSURO BABA, TAKASHI FUKUYAMA, YOSHIKA NAGATA, TETSUYA SO, YOSHINOBU ICHIKI, MASAKAZU SUGAYA, MANABU YASUDA, TOMOKO SO, MITSUHIRO TAKENOYAMA, KENJI SUGIO and KOSEI YASUMOTO

Second Department of Surgery, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, 807-8555, Japan

Abstract. Background: Tumor-infiltrating B lymphocytes (TIB) are often observed in lung cancer. The role of TIB in tumor growth has not been well investigated. Materials and Methods: Forty-four surgically-resected human lung cancer tissues were xenotransplanted into SCID mice. Their blood was collected and the volume of the transplanted tumors was measured regularly. The correlations between the IgG titer in the sera and the growth of the transplanted tumors according to the clinicopathological variables were examined. Results: Human IgG production from TIB was observed in all xenotransplanted mice. Twenty-seven out of the 44 tumors regressed gradually. The average serum human IgG level of the tumor regressors (n=10) was significantly higher than that of the progressors (n=9) in squamous cell carcinoma (p=0.02), while there was no significant difference in the other histological groups. Conclusion: IgG produced by TIB might play a crucial role in preventing tumor growth in squamous cell carcinoma.

It is well accepted that tumor-infiltrating T lymphocytes (TIL) and tumor-associated macrophages (TAM) regulate the tumor-host relationship in various tumors (1). The functions of TIL have been well investigated (2). TIL recognize tumor-associated antigens and numerous tumor antigens have been identified by using TIL (3, 4). Adoptive immunotherapy using TIL showed successful clinical responses (5). In contrast to TIL, there is still little information available about the role of tumor-infiltrating B lymphocytes (TIB), because of the difficulty of maintaining these cells in vitro (6).

Williams et al. engrafted fresh lung cancer tissue specimens into severe combined immunodeficient (SCID) mice and thereby showed the production of human IgG by TIB as well as the recognition of tumor-associated proteins by IgG (7). Lung cancer tissues were engrafted into SCID mice and then IgG from TIB was used as a screening probe for SEREX screening to identify tumor antigens. Twenty-seven genes encoding tumor-associated antigens were identified using this method (8).

However, the effect of such antibodies on the growth of tumors has not yet been elucidated. In this study, the correlation between the amount of IgG produced by TIB and the growth patterns of tumors (i.e., regression or progression) was analyzed.

The study protocol was approved by the Human and Animal Ethics Review Committee of the University of Occupational and Environmental Health, Japan, and written consent was obtained from each patient regarding the use of surgical samples.

Materials and Methods

Patients. Samples were obtained from 44 patients with primary lung cancer who had undergone surgery at our department from September 1996 to August 2004. The eligibility criteria for the patients who entered this experiment were that all had received no anticancer treatment before surgery and all were free from any infectious disease.

Animals. Female SCID mice (BALB/c•C57BL/Ka-Igh-1b/ICR[N17F34] scid/scid, 6-week-old), were purchased from Charles River Inc. (Tokyo, Japan) and were maintained under specific pathogen-free conditions throughout the study.
Engraftment of human cancer tissues into SCID mice. Fresh lung cancer tissues were obtained from surgically-resected specimens. Immediately after resection, the peripheral part of the viable tumor tissue was cut into a block measuring about 5x5x5 mm in order to avoid contamination by necrotic tissue, which is usually located in the central part of tumors. The resected specimens were then engrafted subcutaneously into the bilateral flanks of SCID mice. Blood samples were obtained by a retro-orbital venipuncture from the engrafted SCID mice every 2 or 3 weeks. Therefore, serum samples were collected and frozen at −20°C for analysis of human IgG. The human IgG level was measured by an enzyme immunoassay. The volume of the tumor (V) was calculated based on the following formula; V=0.4πr²h (at the start time; v=50 mm³), where a is the maximum diameter of the tumor, and b is the diameter at a right angle to a (9). Regression or progression of the xenotransplanted tumor was determined 8 weeks after engraftment.

Immunohistochemical staining for B lymphocytes. The immunohisto-chemical detection of B lymphocytes infiltrating into the tumor tissue was performed using peroxidase conjugated mouse monoclonal anti-human CD20 antibody (L26, DAKO Japan Co., Tokyo, Japan). The formalin-fixed paraffin-embedded tissue sections were deparafinized, dehydrated and incubated with 3% hydrogen peroxide in distilled water for 5 min. They were rinsed with distilled water and placed in Tris-buffered saline (TBS) for 5 min and were then incubated with L26 diluted 1:50 for 60 min at room temperature and the Labeled Streptavidin Biotin kit (DAKO Japan Co., Kyoto, Japan) was employed. Thereafter, the slides were washed with water, dehydrated, mounted and observed.

Statistical test. The Student’s t-test was used to determine any significant differences between the two groups. The findings were considered to be significant if the p value was less than 0.05.

Results

Infiltration of B lymphocytes into lung cancer tissue. To confirm the presence of TIB infiltration in the tissue specimens, human lung cancer tissue specimens were stained with hematoxylin eosin and anti-CD20 antibody. One representative case of squamous cell carcinoma (Case No. 25 in Table I), with marked mono-nuclear cell infiltration, is illustrated in Figure 1a. Immunohistochemical staining of the same specimen with anti-CD20 antibody is shown in Figure 1b. Most of the mononuclear cells were found to be CD20-positive B cells.

Correlation between the clinical features of patients and the amount of IgG produced. The clinical characteristics of the 44 patients, whose tumors had been engrafted into the SCID mice, are shown in Table I. The levels of human IgG had been previously reported to reach a peak approximately 6 weeks after engraftment, gradually decreasing thereafter (14). Based on this experience, the representative human IgG level in each case was measured from 5 to 7 weeks after transplantation. Human IgG was detected in all the SCID mice. The average level was 655.9±100.3 µg/ml in all cases (range=11 µg/ml ~2500 µg/ml). The patients were pathologically staged as: stage I, 15; stage II, 10; and stage III, 19. The average serum human IgG levels were 710.4±202.4 µg/ml in stage I, 468.3±108.5 µg/ml in stage II
and 711.7±161.6 μg/ml in stage III. There was no significant difference in the human IgG levels among the pathological stages. Histologically, the 44 tumors were: adenocarcinoma in 21 patients, squamous cell carcinoma in 19, large cell carcinoma in 3 and pleomorphic carcinoma in 1. The average human IgG level was 567.3±115.1 μg/ml in adenocarcinoma and 610.8±154.1 μg/ml in squamous cell carcinoma. No significant difference was observed in the human IgG levels between adenocarcinoma and squamous cell carcinoma. Regression of the engrafted tumor was observed in 27 cases (61.4%), while progression was seen in 17 cases (38.6%).

**Correlation between the amount of IgG produced and the status of tumor growth.** Tumor regression occurred in 15 (71.4%) of the adenocarcinomas and in ten (52.6%) of the squamous cell carcinomas (Figure 2). The average serum human IgG level of squamous cell carcinomas was 939.7±247.3 μg/ml in the tumor regression group and 245.4±68.5 μg/ml in the tumor progression group, representing a statistical difference ($p=0.020$). However, no such significant difference was observed in the adenocarcinomas between regressors and progressors. The time-course in terms of the relationship between tumor growth and the IgG level of representative cases of the tumor regressors and progressors in squamous cell carcinomas is illustrated in Figure 3. The human IgG levels reached as high as 412 μg/ml at 7 weeks after transplantation and the engrafted tumor exhibited progressive growth (Figure 3a). In contrast, the human IgG levels reached a maximum of 1670 μg/ml at 5 weeks after xenotransplantation and the engrafted tumor had regressed completely at 8 weeks after xenotransplantation (Figure 3b).
Discussion

Most tumors have immunogenicity in animals and humans and cell-mediated and humoral immune responses affect the growth patterns of tumors. Using a microcytotoxicity assay, Hellstrom et al. and Yasumoto et al. reported that sera from cancer patients containing cancer-reactive antibodies could strengthen or block cell-mediated cytotoxicity against cancer cells (10, 11). Studies of hybridomas derived from the B lymphocytes of cancer patients indicated the existence of humoral immunity against such tumors (12). Recently, SEREX analysis showed that humoral immune responses to a wide variety of cancer antigens exist in many cancer patients (8, 13-16).

TIB have been hypothesized to have specific functions in antitumor immunity. Several investigators have shown that the IgG antibody produced by TIB has the ability to recognize tumor-associated antigens. Shikola et al. successfully made hybridomas from TIB in glioma which produced an antibody reactive with the tumors (12). This finding suggested that B lymphocytes, specifically sensitized by the tumor, infiltrated glioma tissue. Punt et al. successfully expanded the B lymphocytes in human tumor specimens and malignant effusions using a transformed T cell line MOT as feeder cells and also showed the production of antibody reactive with autologous tumor cells (6). Using a SCID mouse model, Williams et al. and our group reported that plasma cells within a lung tumor xenograft secreted IgG reactive with tumor-associated proteins (7, 8). This system enabled us to evaluate the function of TIB without disrupting the tumor/TIB microenvironment.

Some antibodies are believed to augment cell-mediated cytotoxicity, while others might block such an effect. As shown in Figure 2, a higher level of human IgG reflected tumor regression and a lower level resulted in tumor progression in squamous cell carcinoma of the lung. It remains unclear why only engrafted squamous cell carcinoma regresses significantly while receiving a high level of human IgG. Ito et al. reported that the Th1-to-Th2 ratio in the TIL of lung cancer was significantly elevated in patients with squamous cell carcinoma (17). Using in situ hybridization, Vitolo et al. demonstrated that tumor-infiltrating lymphomononuclear cells showed a greater production of various cytokines, such as the Th1 and Th2 cytokines, especially in squamous cell carcinomas as compared to adenocarcinomas (18). The increased number of Th1 cells were thus considered to activate the Fc receptor-bearing effector cells and such effector cells might eventually kill antibody-binding tumor cells in squamous cell carcinoma.

In this study, it was shown that TIB-derived IgG may play a protective role in tumor progression in squamous cell carcinoma. We will attempt to identify antigens recognized by TIB-derived IgG, especially in squamous cell carcinoma of the lung and also clarify which antigens could induce an antibody to prevent or promote the growth of a transplanted tumor.

Acknowledgements

We thank Miss Kahoru Noda, Yukiko Goto, Yukari Oshibuchi and Mrs. Ayako Yamazaki for their technical expert help and we appreciate the assistance of Mr. Brian Quinn in marking critical points.
References


Received February 28, 2006
Accepted April 4, 2006