T-helper (Th) 1/Th2 Imbalance in the Peripheral Blood of Dogs with Malignant Tumor

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Abstract: T helper type 1 cell (Th1)/Th2 imbalance has been observed in a variety of pathological conditions, including malignant diseases. We evaluated the Th1/Th2 in peripheral blood Th cells by means of intracytoplasmic cytokine analysis in 11 dogs with advanced malignant tumor; four of them showed metastatic tumor. The percentage of Th1 was significantly lower and the percentage of Th2 was significantly higher in diseased dogs compared to healthy dogs. The percentage of Th1 in three patients with metastatic tumor was significantly lower than that in the patients with non-metastatic tumor. We conclude that the Th1/Th2 balance was polarized to Th2 in dogs with cancer.

Key words: Dog, Malignant tumor, Th1/Th2 imbalance

T helper type 1 cells (Th1) and Th2 play important roles on the immunoregulation (1, 8). Th1 promote the cellular immunity through the production of IFN-γ and IL-2 (1). Th2 suppress cellular immunity through the production of IL-4 and IL-10. It has been reported that the imbalance of Th1 and Th2 related with various pathological conditions (1). Recent studies in human have demonstrated that an elevated level of Th2 cytokines may contribute to the escape of tumor cells from the immunosurveillance (5, 9). However, the condition of Th1 and Th2 in dog with malignant tumor has not been reported.

In this study, we evaluated the condition of Th1 and Th2 balance in the peripheral blood of dogs with malignant tumor by using the flow cytometry with the intracytoplasmic cytokine staining method.

A total of 11 dogs with advanced cancer and 10 healthy beagle dogs as control subjects were examined in this study. All patients were histologically diagnosed and had malignant tumor. None of the patients had received any immunosuppressive treatment, including anticancer drug, radiotherapy or corticosteroids. Clinical diagnoses were mammary adenocarcinoma in three, haemangiosarcoma in three, transitional cell carcinoma of the urinary bladder in one, oral melanoma in one, skin melanoma in one, synovial sarcoma in one, and osteosarcoma in one case. A case of mammary adenocarcinoma, a case of osteosarcoma and two cases of haemangiosarcoma showed metastatic tumor.

Heparinized peripheral blood was obtained from both the patients and healthy dogs. Intracytoplasmic cytokine analysis was performed as we have previously described (4). In brief, peripheral blood mononuclear cells (PBMC) were stimulated with a combination of phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich Milwaukee, Wis., U.S.A.), and ionomycin (Sigma-Aldrich) for 4 hr at 37 C in the presence of Brefeldin A (Sigma-Aldrich) for the last 2 hr. After the incubation, non-adherent cells were collected as peripheral blood lymphocytes (PBLs). Then PBLs were stained with fluorescein isothiocyanate (FITC)-conjugated anticanine CD4 monoclonal antibody (mAb: Serotec Raleigh, N.C., U.S.A.). For staining of intracellular cytokine, PBLs were fixed and permeabilized using

Abbreviations: IFN-γ, interferon gamma; IL, interleukin; Th, T helper.
Intraprep (Immunotech Marsaille, France). PBLs were incubated with the respective phycoerythrin (PE)-labeled mAb to each cytokine: IFN-\(\gamma\) as a Th1 cytokine, IL-4 as a Th2 cytokine. Anti-cytokine mAbs used in this study were anti-bovine IFN-\(\gamma\) (Serotec) and anti-bovine IL-4 (Serotec). These subpopulations were analyzed by flow cytometry (FACS Calibar, Becton Dickinson, San Jose, Calif., U.S.A.).

The differences between the two groups were assessed by the Mann-Whitney \(U\) test. \(P\) values less than 0.05 were accepted as statistically significant. All experiments in this study were carried out in accordance with the guidelines for animal experiments issued by the College of Bioresource Sciences, Nihon University.

The percentage of Th1 in the patients (18.70±4.32) was significantly decreased compared with that in healthy dogs (29.91±6.19; \(P<0.01\), Fig. 2). Whereas the percentage of Th2 in the patients (5.14±2.13) was significantly increased compared with that in healthy dogs (3.63±1.43; \(P<0.05\), Fig. 3). The ratio of Th1/Th2 in the patients (4.21±1.86) was significantly lower than that in healthy dogs (9.24±3.48; \(P<0.01\), Fig. 4). These results demonstrates Th1/Th2 imbalance in dogs with malignant tumor and are similar to the result from previous studies with tumor-bearing human (2, 5, 7, 9). Sato et al. used the same methods and reported decreased frequencies of IFN-\(\gamma\)-producing subsets and slightly increased frequencies of IL-4 producing subsets in CD4+ PBMC of various cancers bearing human (9). Cellular immune responses, ranging from dendritic cell maturation to Th1 responses, may be less active in metastasis case than in non-metastasis case in human patients with breast carcinoma before they develop metastasis (10). The up-regulation of Th2 and
regulatory T-cell responses are developed in parallel in metastasis-positive cases (10). Further functional analysis will be needed to design immunotherapy for activation and regulation of metastasis in patients with breast carcinoma. Our results are consistent with their results and the result show Th2 predominance in malignant tumor.

The percentage of Th1 in CD4+ cells in patients with metastatic tumor (14.72 ± 0.98) was significantly decreased compared with that in patients without metastatic tumor (20.97 ± 3.74; *P < 0.05, Table 1). The difference in the percentage of Th2 in CD4+ cells and the mean ratio of Th1/Th2 between patients with metastatic tumor and patients without metastatic tumor was not significant. It may be a trend that patient with metastatic tumor showed more suppressed Th1 immune responses. IFN-γ, which is one of Th1 cytokine, is acting to enhance the recognition of the transformed cell by the immune system. Thus IFN-γ plays a central role in providing an immunocompetent host with a mechanism of tumor surveillance. This suppressed Th1 immune responses may cause the escape of tumor cells from the immunosurveillance and help the development of metastatic tumor.

Although the number analyzed here was still small, our results suggest an imbalance of Th1/Th2 in dogs with malignant tumor. Further studies with a larger number of dogs with malignant tumor are in progress in our institute.

The imbalance of Th1/Th2 observed in this study might be involved in the mechanism that allows the tumor to escape from host immune surveillance. Immunotherapy for cancer may be hopeful if this imbalance can be repaired.

References


