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Reduction of tumor necrosis factor production by splenocytes from v-Ha-ras oncogene-bearing mice

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Abstract

Mice containing the activated v-Ha-ras oncogene driven by the mouse mammary tumor virus promoter/enhancer produced less tumor necrosis factor (TNF) than genetically identical animals without it. Inbred Oncomice containing the v-Ha-ras oncogene and inbred FVB mice without it were grown for 6 months. Splenocytes were isolated and stimulated in vitro to produce tumor necrosis factor (TNF) and γ-interferon (IFN). TNF production by cells from Oncomice was significantly decreased compared to cells from FVB mice. There was a tendency for decrease, but no significant difference was seen on IFN release. These observations suggest that the oncogene may play a role in the immune system.

Key words: Oncomouse; Tumor necrosis factor; γ-Interferon; V-Ha-ras oncogene

1. Introduction

The Oncomouse is an inbred FVB mouse which contains an activated v-Ha-ras oncogene [1]. Due to the oncogene, the Oncomouse predictably undergoes carcinogenesis. Both males and females develop parotid adenocarcinomas and Harderian gland hyperplasia as well as breast carcinomas, although at different rates [1]. Thus, the oncogenes in transgenic mice are a necessary, but not sufficient, condition for the complete manifestation of a tumor. Oncoprotein is required to deregulate the cells [2–3]. The v-Ha-ras oncogene was found to be expressed on mammary, salivary and lacrimal glands as well as on spleen and thymus cells in Oncomice [1]. Therefore, oncogene products are produced by lymphoid cells and could alter their immune functions.

Products of the oncogene might deregulate lymphoid and other cells by altering cytokine production. Tumor necrosis factor (TNF) is a cytokine which is produced by macrophages and T-lymphocytes and has direct antitumor activity [4–8]. In addition, it up-regulates immune responses [9], affects immune cells [9], and determines tumor development. γ-Interferon (IFN) activates natural killer cells with stimulation of antitumor activity. If the presence of the v-Ha-ras
oncogene changed the production of immuno-regulatory cytokines, it could have profound effects on tumor cell growth. Therefore, we measured TNF and IFN production by splenocytes from Oncomice and normal FVB mice in order to evaluate the effect of the presence of v-Ha-ras oncogene on cytokine production in vitro.

2. Materials and methods

2.1. Animals

Female FVB mice were obtained from the National Cancer Institute and female Oncomice were received from the Du-Pont Company. The mice were viral antibody-free as determined by the University of Arizona Animal Care Department (Coronavirus (Mouse Hepatitis Virus), Sendai, Mycoplasma pulmonis). They were received at 3–4 weeks of age. They were maintained at a 12-h light-dark cycle, with food and water supplied ad libitum for 20 weeks.

2.2. Cell Culture

FVB and Oncomice were sacrificed by bleeding from axillary vein under ether anesthesia. Then, sterile single spleen cell suspensions were made in RPMI 1640 with 10% fetal calf serum by teasing the spleen with forceps. The cells at a concentration of 10^6 cells per 100 μl of RPM 1640 with 10% fetal calf serum were laid down into each well of a 96-well plate (Falcon 3072), and another 100 μl of 10 pg/ml lipopolysaccharide (LPS) or 4 pg/ml of Concanavalin A (ConA) were added. The plates were kept at 37°C, 5% CO₂ incubator for 24 h or 72 h. After incubation the plates were centrifuged at 180×g for 10 min and supernatants were collected for detection of TNF or IFN.

ELISA of TNF and IFN

An ELISA system was set up in our laboratory as described previously [10]. Briefly, the 96-well ELISA plates (Dynatech Immunolon 2) were coated with 50 l of Hamster anti-murine TNF monoclonal antibody (Genzyme, Boston, MA) or rat anti-murine IFN (γ-interferon) monoclonal antibody (Lee Molecular Lab, San Diego, CA) diluted 1:500 in carbonate buffer (pH 9.6), and incubated at 4°C overnight. The plates were washed with 0.01 M PBS containing 0.05% Tween 20 (PBS-Tween) and dried. Then 50 μl of standards (recombinant murine TNF or IFN, Genzyme), or 50 μl of supernatants from cell culture were added in each well, incubated in 37°C, 5% CO₂ incubator for 2 h, washed and dried as before. Following drying, 50 μl of rabbit-anti-murine TNF or IFN polyclonal antiserum (diluted 1:167 and 1:100 in PBS-Tween, a kind gift from Dr. Philip Scuderi, Arizona Cancer Center) were placed in each well and incubated for another 2 h at 37°C in a 5% CO₂ incubator. The plates were washed and dried as before, 50 μl of goat anti rabbit IgG conjugated with HRP (American Oualex, 1:5000 diluted in PBS-Tween) were added, and incubated for another 1 h. The plate was washed with PBS-Tween three times and once with PBS. After drying the enzyme substrate, 100 μl of ABTS was added to each well. It contained 0.54 g 2′,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) and 21.0 g citric acid monohydrate in 1 l of water at pH 4.2. Before use, 10 μl of H₂O₂ was added to 10 ml of ABTS. After 30 min, the optical density was measured on a Titertek Multiskan ELISA reader at 405 nm. The standard range for TNF was 0.156–10 ng/ml with a detection limit of 10 pg/well. The standard range for IFN was 0.39–25 ng/ml with a detection limit of 20 pg/well.

3. Results

3.1. Spleen and thymus weight and cell numbers

There was no significant difference in spleen weight and cell numbers between FVB and Oncomice. Thymus weights were the same in the Oncomouse as in the FVB mouse, but, the thymus cell number was significantly (P < 0.05) lower in the Oncomouse than that in the FVB mouse (Table 1).

3.2. Cytokine production

The spleen cells from Oncomice produced significantly less TNF in vitro than those isolated from FVB mice after stimulation by LPS (5 μg/ml) in vitro. Also, IFN production by spleen cells from the Oncomouse had a tendency to lower values when compared to FVB mouse cells which was not statistically significant (Table 2).
Table 1

|                     | FVB Mouse | Oncomouse |
|---------------------|-----------|-----------|
| Spleen weight (mg)  | 152.0 ± 37.0 | 127.0 ± 55.0 |
| Thymus weight (mg)  | 30.15 ± 7.89  | 30.56 ± 2.35  |
| Spleen cell number  | 25.99 ± 9.76  | 20.52 ± 7.48  |
| Thymus cell number  | 7.03 ± 2.34   | 4.45 ± 1.66b |

aData presented are mean ± S.D. with n = 14 for FVB mice and n = 9 for Oncomice.

bSignificant differences between FVB and Oncomice, P < 0.05, using two-tailed Student’s t-test.

4. Discussion

Oncogenes inserted at a very early stage of the development result in all cells in the body bearing the oncogenes, including the cells of the immune system. Thus, transgenic mice are a novel probe for immunological studies, which we report utilizing for the first time. The changes due to oncogenes could disrupt functions and interactions of lymphoid cells in the complex immune system [2]. Some studies suggested that oncogenes do not immediately produce a condition of continuous growth, but rather require a period of time to deregulate the cells [11]. They may allow or induce genetic or epigenetic changes [11]. These changes involve not only gross chromosomal rearrangement, but also occur as deletions or mutations at specific chromosomal loci. The latter changes include inactivation of tumor suppressor genes, the products of which restrict cell proliferation and other aspects of tumor growth [12].

v-Ha-ras oncogenes, driven by the mouse mammary tumor virus promoter, induces mammary gland tumors in Oncomice. These mammary gland tumors have a pre-neoplastic stage of their development [11]. Sugimoto et al. [13] reported that the v-Ha-ras-transformed, murine fibroblast cell line NIH3T3 had a decreased expression of Thy-1 antigen on their surface, which is an important membrane protein in the immune system affecting cell growth. In this study, Oncomice were sacrificed at 24 weeks old, when tumors started to appear [14]. In our experiment, only two Oncomice had visible tumors.

During tumor initiation, cytokines may have major effects on cells including regulation of the whole system for surveillance. TNF is a multifaceted cytokine which regulates immune functions, and plays a major role in anti-tumor activity of host immune defense. Thus, the lower TNF production by Oncomouse spleen cells in vitro observed in our study may reflect the deregulation by oncogenes and contribute to the development of detectable tumors. Although IFN production also has a tendency to lower levels, no statistically significant difference was found. We recognize that there might have been a difference in mitogenesis, reflected in altered cytokine synthesis as we recently showed that retrovirus infected mice had spleen cells which were very unresponsive to mitogens [15]. These cells produced little IFN but large amounts of interleukin 4-6, and TNF. Thus, changes in mitogenesis are unlikely to explain altered TNF secretion seen here.

Therefore, the presence of the v-Ha-ras oncogene on lymphoid cells may favor the growth of v-Ha-ras oncogene-bearing tumors by decreasing the production of cytokines which are vital regulatory components of the immune system. This process is important in immune surveillance against tumor cells by activation of natural killer cells, for tissue macrophages, as well as for direct antitumor activities.
5. Acknowledgments

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6. References

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