Tumor Promoter-Induced Systemic Immunomodulation: Significance in Skin Chemical Carcinogenesis

Hyunah Lee: Division of Hematology/Oncology, Department of Medicine, Samsung Medical Center, Seoul, Korea.

Introduction

Experimental skin cancer can be chemically induced in mice by topical application of subchronic dose of carcinogen (initiation phase) followed by repetitive use of non carcinogenic (or weakly carcinogenic) chemicals termed "tumor promoters" (promotion phase). The process of initiation entails somatic mutations, and the chemicals that function as initiators in skin are polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP) or 7,12-dimethylbenz(a)anthracene (DMBA), whereas the promotion is epigenetic process. Among numerous promoting chemicals known, 12-O-tetradecanoylphorbol-13-acetate (TPA) is the most potent promoters.

Dose response studies indicate that murine strains differ markedly in their sensitivities to TPA in two-stage chemical induced skin carcinogenesis protocol employing DMBA as the initiator and TPA as the promoter. (SSIN > SENCAR > CD-1 > DBA/2 > BALB/c > C57BL/6, B6C3F1) Consequently, comparisons among mice differing in their sensitivities to TPA are commonly used as a tool to assess the role of immunomodulation in the processes of chemical-mediated promotion.

There are two theories proposing how the immune system participates in the processes of chemical-induced skin cancer.

"Immunosurveillance" theory proposes that cancer develops as a consequence of a failure of the immune system to recognize and eliminate an emerging neoplasm. While "immunostimulation/immunofacilitation" theory suggests that under certain conditions the immune system actually contributes to the development of neoplasia. Our systemic immunomodulation studies reported herein demonstrate that both theories are germane to the processes of chemical-induced skin cancer.

I. Differences in Systemic Immunity among Different Strains of Mice.

Systemic immunosurveillance functions (natural killer and cytotoxic T cell activity) were compared in the strains of mice differing sensitivity to two stage chemical carcinogenesis protocol employing DMBA as an initiator, TPA as a promoter. (B6C3F1, C57BL/6, SENCAR and SSIN)

Without application of TPA, single cell suspension of splenic leukocytes from each strain of mice was prepared as effector cells in natural killer (NK) activity. Radioactivity released from the coculture of 51Cr-labeled YAC-1 mouse lymphoma cells and effector cells was counted as specific NK activity. On E:T ratio of 50:1, % specific NK activity was 9.06$\pm$0.61, 4.17$\pm$0.52, 3.76$\pm$0.86, and 2.63$\pm$0.41 for B6C3F1, C57BL/6, SENCAR and SSIN mice, respectively. Splenic NK activity in B6C3F1 mice was significantly higher than any other strains of mice.

Naive mice of each strain were injected intraperitoneally (i.p.) with allogeneic P815 mouse mastocytoma cells from DBA/2 to induce cytotoxic T lymphocytes. Induced CTLs in spleen were prepared as single cell suspension to serve as effector cells in CTL activity test using 51Cr-labeled P815 cells as target cells. On E:T ratio of 25:1, % cytotoxicity in different

strains of mice were 15.69±1.31, 13.44±1.34, 11.27±0.68, and 6.95±2.10 for C57BL/6, B6C3F1, SENCAR and SSIN, respectively. CTL activity in SSIN was significantly lower than any other strain of mice.

Analysis of splenic T cell subpopulation by flow cytometry revealed that the unusually low proportion of CD8+ T cells in SSIN mice compared to the other strains, suggesting the possibility of genetic deficiency of CTLs in SSIN. The ratios of CD4+:CD8+ were 4.3:1, 2.9:1, 1.8:1, and 1.7:1 in SSIN, SENCAR, B6C3F1 and C57BL/6, respectively.

These results demonstrated the inverse-proportion between sensitivity to DMBA-TPA induced two-stage skin carcinogenesis protocol and systemic cellular immunity (NK & CTL activity). Therefore, these data may support the immunosurveillance theory involved in chemical-induced skin carcinogenesis process.

II. Systemic immunomodulation by topically applied tumor promoter, TPA in SENCAR and B6C3F1 mice.

The nonspecific cytotoxic activity against tumor cells (NK activity) was suppressed in tumor promoter-sensitive SENCAR, while enhanced in promoter-resistant B6C3F1 mice by topical application of TPA. This differential modulation of splenic NK activity by topically applied TPA in the SENCAR, and B6C3F1 mice supported the immunosurveillance theory. Splenic T cell mitogenesis induced by PHA and the percentage of T cells (Thy1.2) was decreased after TPA topical application in both SENCAR and B6C3F1 mice.

Topically applied TPA evoked significantly greater dermal inflammatory responses in SENCAR than in B6C3F1 mice. Along with

differential inflammatory responses on the skin, increases in the splenic nucleated cell numbers (specifically, the leukocytes lacking both T and B cell markers, presumably granulocyte-macrophage progenitor cells), and serum granulocyte-macrophage colony stimulating activity (GM-CSA) by TPA were greater in SENCAR mice than in B6C3F1 mice. TPA-induced greater induction of superoxide anion production in peritoneal macrophages (MPs) from SENCAR and above data suggested the involvement of stimulated immunoinflammatory responses in tumor promotion.

III. TPA-induced systemic immunomodulation in SSIN mice

SSIN is the SENCAR inbred that posses the super sensitivity to DMBA-TPA induced two-stage chemical skin carcinogenesis protocol. Dermal inflammatory response after topical application of TPA was greater than in SENCAR. As shown above, along with unusually low CD8+ T cell proportion in the spleen, the nonspecific (NK) or antigen-specific (CTL) cytotoxic activity against tumor cells in SSIN mice were much lower than in SENCAR. Concomitantly, compared to SENCAR, topical applications of TPA modulated systemic immunity of SSIN differentially.

The increase of splenic nucleated cell number was not significant after topical application of TPA (2ug TPA /application, twice/week, 2-4weeks). Unlike the case in SENCAR, mitogen (ConA)-induced T cell proliferation was increased (374 - 479 % increase compared to naive control). LPS-induced B cell proliferation was increased (430 - 571 % increase compared to naive control) by TPA, as well. Modulation of NK or CTL activity by topical application of TPA was not significant in SSIN. Infact, slight increase in p815 cell-induced CTL activity was observed after

chronic application (4weeks application of 2ug TPA/application).

Humoral immunity modulated by topical application of TPA was determined by plaque forming cell (PFC) assay against T cell-dependent antigen, sheep red blood cell (SRBC). Antibody production after first immunization with SRBC (IgM) was inhibited by 2 weeks application of TPA, whereas 4 weeks application of TPA enhanced the production of IgM. Production of IgG, antibody produced after second immunization, was not significantly modulated by TPA. TPA-induced modulation of antibody production was clearly T cell-dependent phenomenon as was tested with TNP-LPS, a T cell-independent antigen.

Flow cytometric analysis of splenocytes from SSIN mice, topically applied with TPA revealed the significant decreases in the proportion of T cells ($CD3^+$ as well as $CD4^+$). On the contrary, functioning of T cells was enhanced as was shown in mitogenesis against ConA. The proportion of $CD8^+$ cells in naive control SSIN mice was unusually low, and 2 week application of TPA significantly down modulated it.

Unlike in SENCAR, TPA up regulated or non significantly modulated the many of systemic immunity including CTL activity, NK activity, and T and B cell mitogenesis in SSIN mice. According to immunosurveillance theory, these immunomodulatory responses did not explain the super sensitivity of SSIN mice in two-stage chemical carcinogenesis protocol.

Conclusion

The role of immunomodulation in the mechanism of promotion by chemicals has not been seriously considered. However, the studies clearly

demonstrated that topically applied TPA is the potent immunomodulator affecting both local and systemic *in vivo* processes . The question asking how this immunomodulations contribute to the promotion process has not been clearly answered. The results in this presentation demonstrated that the TPA-induced immunomodulation involves in the processes of both "immunosurveillance" and "immunostimulation/immunofacilitation"