A cancer immunosurveillance controversy

To the editor:

Recently, the idea of T cell–mediated cancer immune surveillance against chemically induced and spontaneous cancer was revived[1], based on studies showing that mice lacking IFN-γ, IFN-γ-receptor (IFN-γR), IFN-γ-producing cell types or perforin have increased tumor susceptibility to 3-methylcholanthrene (MCA) and a high tumor incidence in aged mice deficient in recombination activation gene 2 (RAG-2). All further experiments were based on tumor transplantation. It is unlikely that rejection of transplanted tumor cells, typically mediated by T cells, and inhibition of MCA carcinogenesis involve the same mechanism. We suggest that a 'tissue repair' response, during which MCA is encapsulated[2], may mediate protection. In contrast to immune surveillance, a tissue repair response does not involve T cells. It is suspicious that regardless which IFN-γ producers ($\alpha\beta^+$, $\delta\gamma^+$ or all T cells; natural killer or natural killer T cells) are lacking, mice have increased susceptibility to MCA[1]. This indicates that either all these cells are active participants during tumor elimination[1] or steady-state IFN-γ concentrations are different between the knockout and control mice. In most of the studies mentioned above[1] the immune-deficient mice were bred locally and control mice were purchased. The transfer of mice to a new environment or a local T cell response induces widespread IFN-γ-dependent major histocompatibility complex expression in remote organs[3,4]. Therefore, exposure of mice to a new environment with differences in flora, food, air and water would probably influence the immune status and increase IFN-γ production. This could nonspecifically contribute to the protective response against MCA. Thus, use of non-littermates, the transfer of mice to a new environment or unknown environmental factors in animal facilities could increase IFN-γ production by control mice in a manner unrelated to the experimental treatment.

Therefore, we reinvestigated MCA carcinogenesis using RAG-1-deficient mice and perforin-deficient mice. Littermates or knockout and wild-type mice obtained from the same colony were used as controls. Both RAG-1-deficient and control mice developed tumors at similar frequencies; the T cell–competent group showed only a slight delay (**Fig. 1a**). We obtained similar results with other T cell–deficient mice (data not shown). Perforin-deficient mice also developed tumors with the same kinetics and incidence as control mice (**Fig. 1b**). Thus, in contrast to IFN-γR-deficient animals[2], mice lacking only some IFN-γ-expressing cell types or perforin do not show substantially increased susceptibility to MCA. This argues against the idea of immune surveillance of MCA-induced carcinogenesis mediated by IFN-γ-producing T cells.

The development of spontaneous tumors in aged life-long immunosuppressed RAG-2- and IFN-γ-deficient mice[5,6] as evidence of immune surveillance should be interpreted with great caution, because opportunistic infection and chronic inflammation can be associated with tumor development[7]. For example, spontaneous tumor development in IFN-γ-deficient mice can be prevented with antibiotics[8], and aged RAG-2-deficient mice do not develop colon carcinomas unless infected with helicobacter[9]. Therefore, tumor development in immunosuppressed mice does not necessarily indicate that tumor development was similar and controlled by T cells in wild-type mice.

The correlation between prolonged MCA-induced tumor latency in wild-type mice compared with RAG-2-deficient mice was explained by T cell–mediated selection of less immunogenic tumors. However, no such correlation was noted in comparable experiments using IFN-γR-deficient[3] and severe combined immunodeficient mice[10]. Given the evidence discussed showing T cells do not directly protect against MCA-induced carcinogenesis, it is unlikely that T cell–mediated immune selection and escape can explain latency in MCA-treated mice. Thus, new evidence supporting the idea of T cell–mediated immunosurveillance against chemical carcinogen-induced or spontaneous cancer is lacking.

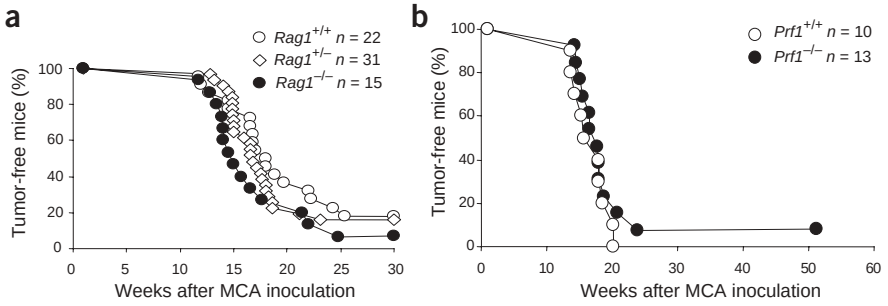

Figure 1 RAG-1- and perforin-deficient mice have tumor incidence similar to that of wild-type mice after injection of MCA. (**a**) RAG-1-deficient (*Rag1*$^{-/-}$) and heterozygous (*Rag1*$^{+/-}$) and homozygous (*Rag1*$^{+/+}$) control littermates bred at our institute (originally obtained on a C57BL/6 genetic background) were injected intramuscularly with 25 μg MCA in 0.1 ml sesame oil, and tumor development was monitored. Mice bearing a tumor ≥1 cm in diameter were considered tumor-positive. Similar results were obtained in a second experiment and in experiments using severe combined immunodeficient and nude mice. (**b**) C57BL/6 perforin-deficient (*Prf1*$^{-/-}$) and wild-type (*Prf1*$^{+/+}$) mice obtained from the Jackson Laboratories were injected intramuscularly with 25 μg MCA as described above. Similar results were obtained when 100 μg was injected intramuscularly, 50 μg was injected subcutaneously or 25 μg was injected subcutaneously into perforin-deficient mice and control littermates bred at our institute.

For the complete version of this correspondence, see **Supplementary Note 1** on the *Nature Immunology* website.

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Schreiber *et al.* reply:

Qin and Blankenstein argue that there is insufficient evidence to revive the cancer immunosurveillance hypothesis. However, three lines of evidence strongly support its existence in mice[1] (**Supplementary Table 1** online). Specifically, immunocompromised mice develop more carcinogen-induced tumors than do immunocompetent mice; carcinogen-induced tumors from immunodeficient mice are more immunogenic than those from immunocompetent mice; and immunocompromised mice develop more spontaneous tumors and lymphomas than do normal mice.

In contrast to others, Qin and Blankenstein did not find increased incidence of MCA-induced tumors in mice lacking RAG-1 or perforin (*Prf1*) compared with wild-type mice. However, they did not do the appropriate dose-response experiments, because they used MCA doses that induced tumors in nearly all the immunocompetent mice (**Fig. 1**). This probably overwhelmed the protective effects of immunity, just as high virus doses obfuscate differences in disease susceptibility between immunocompetent and immunodeficient hosts. Qin and Blankenstein also administered MCA intramuscularly rather than using the conventional subcutaneous route, which could change the pharmacokinetics of MCA, the tumor microenvironment and, potentially, the *in vivo* biological potency of MCA. Finally, tumor incidences were not monitored separately in male versus female mice, which have different susceptibility to MCA.

Qin and Blankenstein argue that our MCA results can be explained by genetic or environmental differences between wild-type and immunodeficient mice. Thus, they dismiss extensive data from several groups[1] (**Supplementary Table 1** online) showing that 11 different types of immunodeficient, gene-targeted mice on four distinct genetic backgrounds showed increased tumor incidences compared with wild-type counterparts. Qin and Blankenstein use a recent publication to argue that comparisons between wild-type and knockout mice are invalid because of uncontrolled epigenetic alterations in knockout mice. However, a subsequent paper reported that epigenetic abnormalities in embryonic stem cells and chimeras are not transmitted to progeny[2], thus undermining this argument. Qin and Blankenstein also suggest that environmental differences between knockout and wild-type mouse colonies contribute to the increased susceptibility to carcinogens of the former. However, we used control and gene-targeted mice generated and housed in the same facilities. Moreover, normal mice rendered immunodeficient by antibody treatment were genetically identical and were derived from identical sources as those left untreated. Finally, the results obtained were consistent between several independent laboratories. Thus, the differences we observed in MCA tumor formation can only be explained by the presence or absence of an intact immune system.

Qin and Blankenstein agree with us that endogenously produced IFN-γ prevents MCA tumor induction, but they propose that IFN-γ stimulates a 'foreign body' reaction that encapsulates MCA, limiting its transforming activity. However, no data exist showing that MCA encapsulation inhibits tumorigenesis. Indeed, encapsulation could just as well concentrate the carcinogen, thereby improving its transforming activity, but this process is balanced by the immune elimination of tumors in immunocompetent mice. Moreover, encapsulation cannot explain why *Prf1*$^{-/-}$ mice[3,4] or mice lacking tumor necrosis factor–related apoptosis-inducing ligand (TRAIL)[5,6] show increased MCA tumor incidence similar to that of mice lacking IFN-γ or IFN-γ responsiveness. In fact, existing data support a broader model whereby IFN-γ coordinates both immune and nonimmune processes resulting in tumor destruction[1,7]. For example, IFN-γ affects tumors directly by enhancing their MHC class I antigen processing and presentation functions, thus facilitating recognition and elimination by CD8$^+$ T cells and by stimulating production of angiostatic chemokines (IP-10 and MIG) that also promote tumor death. At the host level, IFN-γ induces expression of the TRAIL on NK cells, facilitates tumor-specific CD8$^+$ T cell generation and stimulates stromal cell production of angiostatic factors in the tumor microenvironment.

Qin and Blankenstein criticize the tumor transplantation approaches we used to complement the primary tumorigenesis experiments. However, transplantation is the only method to assess comparative immunogenicities of carcinogen-induced tumors. These studies showed that MCA-induced tumors from lymphocyte-deficient mice are more immunogenic than those from immunocompetent mice, demonstrating immunity's dual function in protecting the host against tumor development while also selecting for tumor cells with lowered immunogenicity, a process that has been called 'cancer immunoediting'[1,7].

Qin and Blankenstein claim that they did not find enhanced immunogenicity in MCA-induced tumors from immunocompromised mice. However they compared the immunogenicities of tumor cells from IFN-γR-deficient mice with those from wild-type mice. Tumor cells from IFN-γR-deficient mice have an intrinsic defect in expressing their immunogenicity because of their IFN-γ insensitivity and thus are unsuitable for use in these comparisons[1,7].

Qin and Blankenstein argue that the increased occurrence of spontaneous tumors in immunodeficient mice results from persistent microbial infection. They refer to work showing that spontaneous tumor formation in mice lacking both IFN-γ and granulocyte-monocyte colony-stimulating factor can be prevented by antibiotics[8] and that aged RAG-2-deficient mice do not develop colon carcinomas unless infected with helicobacter[9]. However, the aged mice used in our studies were maintained on the same broad-spectrum antibiotic used in the IFN-γ–granulocyte-monocyte colony-stimulating factor study, were consistently found to be negative for multiple *Helicobacter* strains and showed no idiopathic intestinal inflammation[7]. Nevertheless, it is possible that the immune system functions both to