(Sigma), mTREM-1/IgG1, hILT-3/IgG1[16], or heat-inactivated mTREM-1/IgG1 (30 min, 95 °C) by i.p. injection at 1, 2, 4 and 6 h after or 1 h before LPS administration. We monitored viability of treated mice 4–6 times a day for at least 10 days.

Analysis of blood and peritoneal lavage

Blood (250 μl) was collected and serum TNF-α and IL-1β were determined using cytokine-specific enzyme-linked immunosorbent assays as per the manufacturer's protocol (R&D Systems). Total cell numbers of peritoneal lavage cells collected from treated and control mice were determined on a coulter counter. We performed differential counts according to standard morphological criteria on cytospin preparations stained with Giemsa/May-Grünwald solution (Sigma). Four-colour analysis of peritoneal leukocytes was performed after blocking Fc receptors (FcR blocking agent; Pharmingen) for 30 min, using anti-mTREM-1, anti-Ly-6G (Pharmingen), anti-Mac-1 (Pharmingen) monoclonal antibodies conjugated with APC, PE and FITC, respectively. Dead cells were excluded by staining with propidium iodide.

Escherichia coli peritonitis model

E. coli peritonitis was induced in mice as described[25]. Briefly, female C57BL/6 mice (8–10 weeks, 19–22 g) were weighed and randomly distributed into groups of 5–15 animals of equal body weight. Mice were injected i.p. with 500 μg of mTREM-1/IgG1 or control hIgG1 before i.p. administration of 500 μl of a suspension of *E. coli* O111:B4 ($1.6–2.1 \times 10^6$ colony forming units per mouse).

Caecal ligation and puncture

We performed CLP as described[17,24]. Briefly, female C57BL/6 mice (8–10 weeks, 19–22 g) were anaesthetized by i.p. administration of 75 mg per kg Ketanest (Parke Davies) and 16 mg per kg Rompun (Bayer AG) in 0.2 ml sterile pyrogen-free saline (B. Braun Melsungen AG). The caecum was exposed through a 1.0–1.5-cm abdominal midline incision and subjected to a 50–80% ligation of the distal half followed by a single puncture with a G23 needle. A small amount of stool was expelled from the punctures to ensure patency. The caecum was replaced into the peritoneal cavity and the abdominal incision closed in layers with 5/0 Prolene thread (Ethicon). Sterile saline (500 μl) containing 500 μg mTREM-1/IgG1, 500 μg hIgG1κ (Sigma) or 100 μg TNF-RI/IgG1 (Pharmingen) (together with 400 μg hIgG1κ; Sigma) was administered by i.p. injection immediately after CLP. The CLP was performed blinded to the identity of the treatment group. We assessed survival after CLP 4–6 times a day for at least 7 days.

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1. Medzhitov, R. & Janeway, C. Jr Innate immunity. *N. Engl. J. Med.* **343,** 338–344 (2000).
2. Hoffmann, J. A., Kafatos, F. C., Janeway, C. A. & Ezekowitz, R. A. Phylogenetic perspectives in innate immunity. *Science* **284,** 1313–1318 (1999).
3. Aderem, A. & Ulevitch, R. J. Toll-like receptors in the induction of the innate immune response. *Nature* **406,** 782–787 (2000).
4. Beutler, B. Endotoxin, toll-like receptor 4, and the afferent limb of innate immunity. *Curr. Opin. Microbiol.* **3,** 23–28 (2000).
5. Bone, R. C. The pathogenesis of sepsis. *Ann. Intern. Med.* **115,** 457–469 (1991).
6. Beutler, B., Milsark, I. W. & Cerami, A. C. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science* **229,** 869–871 (1985).
7. Morrison, D. C. & Ryan, J. L. Endotoxins and disease mechanisms. *Annu. Rev. Med.* **38,** 417–432 (1987).
8. Tracey, K. J. *et al.* Shock and tissue injury induced by recombinant human cachectin. *Science* **234,** 470–474 (1986).
9. Glauser, M. P., Zanetti, G., Baumgartner, J. D. & Cohen, J. Septic shock: pathogenesis. *Lancet* **338,** 732–736 (1991).
10. Bouchon, A., Dietrich, J. & Colonna, M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J. Immunol.* **164,** 4991–4995 (2000).
11. Lanier, L. L., Corliss, B. C., Wu, J., Leong, C. & Phillips, J. H. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature* **391,** 703–707 (1998).
12. Wang, H. *et al.* HMG-1 as a late mediator of endotoxin lethality in mice. *Science* **285,** 248–251 (1999).
13. Bernhagen, J. *et al.* MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature* **365,** 756–759 (1993).
14. Ohlsson, K., Bjork, P., Bergenfeldt, M., Hageman, R. & Thompson, R. C. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* **348,** 550–552 (1990).
15. Alexander, H. R., Doherty, G. M., Buresh, C. M., Venzon, D. J. & Norton, J. A. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. *J. Exp. Med.* **173,** 1029–1032 (1991).
16. Cella, M. *et al.* A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. *J. Exp. Med.* **185,** 1743–1751 (1997).
17. Echtenacher, B., Falk, W., Mannel, D. N. & Krammer, P. H. Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J. Immunol.* **145,** 3762–3766 (1990).
18. Echtenacher, B., Mannel, D. N. & Hultner, L. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* **381,** 75–77 (1996).
19. Malaviya, R., Ikeda, T., Ross, E. & Abraham, S. N. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α. *Nature* **381,** 77–80 (1996).
20. Rothe, J. *et al.* Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF- mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature* **364,** 798–802 (1993).
21. Pfeffer, K. *et al.* Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* **73,** 457–467 (1993).
22. Peschon, J. J. *et al.* TNF receptor-deficient mice reveal divergent roles for p55 and p75 in several models of inflammation. *J. Immunol.* **160,** 943–952 (1998).
23. Eskandari, M. K. *et al.* Anti-tumor necrosis factor antibody therapy fails to prevent lethality after cecal ligation and puncture or endotoxemia. *J. Immunol.* **148,** 2724–2730 (1992).
24. Calandra, T. *et al.* Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nature Med.* **6,** 164–170 (2000).
25. Appelmelk, B. J. *et al.* Use of mucin and hemoglobin in experimental murine gram-negative bacteremia enhances the immunoprotective action of antibodies reactive with the lipopolysaccharide core region. *Antonie Van Leeuwenhoek* **52,** 537–542 (1986).
26. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit. Care. Med.* **20,** 864–874 (1992).
27. Facchetti, F. *et al.* Suppurative granulomatous lymphadenitis. Immunohistochemical evidence for a B-cell-associated granuloma. *Am. J. Surg. Pathol.* **16,** 955–961 (1992).

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IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity

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Lymphocytes were originally thought to form the basis of a 'cancer immunosurveillance' process that protects immunocompetent hosts against primary tumour development[1,2], but this idea was largely abandoned when no differences in primary tumour development were found between athymic nude mice and syngeneic wild-type mice[3–5]. However, subsequent observations that nude mice do not completely lack functional T cells[6,7] and that two components of the immune system—IFNγ[8,9] and perforin[10–12]—help to prevent tumour formation in mice have led to renewed interest in a tumour-suppressor role for the immune response. Here we show that lymphocytes and IFNγ collaborate to protect against development of carcinogen-induced sarcomas and spontaneous epithelial carcinomas and also to select for tumour cells with reduced immunogenicity. The immune response thus functions as an effective extrinsic tumour-suppressor system. However, this process also leads to the immunoselection of tumour cells that are more capable of surviving in an immunocompetent host, which explains the apparent paradox of tumour formation in immunologically intact individuals.

Age-matched female wild-type mice and immunodeficient mice with a targeted disruption of the recombination-activating gene-2 (RAG2) that is expressed only in lymphocytes[13], both on a pure 129/SvEv genetic background, were injected subcutaneously with 100 μg of the chemical carcinogen methylcholanthrene (MCA) and monitored for tumour development. $RAG2^{-/-}$ mice developed tumours earlier than wild-type mice and with greater frequency ($P < 0.01$). After 160 days, 9/15 $RAG2^{-/-}$ mice but only 2/15 wild-type mice formed MCA-induced tumours (Fig. 1a). Similar results

were obtained in two additional experiments. In total, 30/52 RAG2$^{-/-}$ mice formed MCA-induced tumours compared to only 11/57 wild-type mice (Fig. 1b) ($P < 0.0001$). The increased tumour formation observed in RAG2$^{-/-}$ mice was comparable to that observed in 129/SvEv strain, IFNγ-insensitive mice that lack either the IFNγ receptor (IFNGR1) or STAT1, the transcription factor that plays a dedicated role in mediating signalling by the IFNγ and IFNα/β receptors[14,15] (12/20 and 17/30, respectively, versus 11/57 wild-type mice; $P < 0.001$); this data is consistent with our previous findings[9] (Fig. 1b). To assess the extent of collaboration between the lymphocyte- and IFNγ/STAT1-dependent tumour-suppressor mechanisms, we generated RAG2$^{-/-}$ × STAT1$^{-/-}$ mice (RkSk mice) and assessed their MCA sensitivity. Mice lacking both genes also showed increased susceptibility to MCA-induced carcinogenesis with 13/18 mice forming tumours compared to 11/57 wild-type mice ($P < 0.0001$), but did not display a significantly greater tumour incidence compared to mice that lacked either the RAG2 or STAT1 genes individually (Fig. 1b) ($P = 0.127$ and 0.140, respectively). All four groups of mice formed histologically indistinguishable sarcomas at the injection site. Similar results were obtained using male mice, which are known to be more susceptible to MCA-induced tumorigenesis. Thus, T, NKT and/or B cells are essential to suppress development of chemically induced tumours.

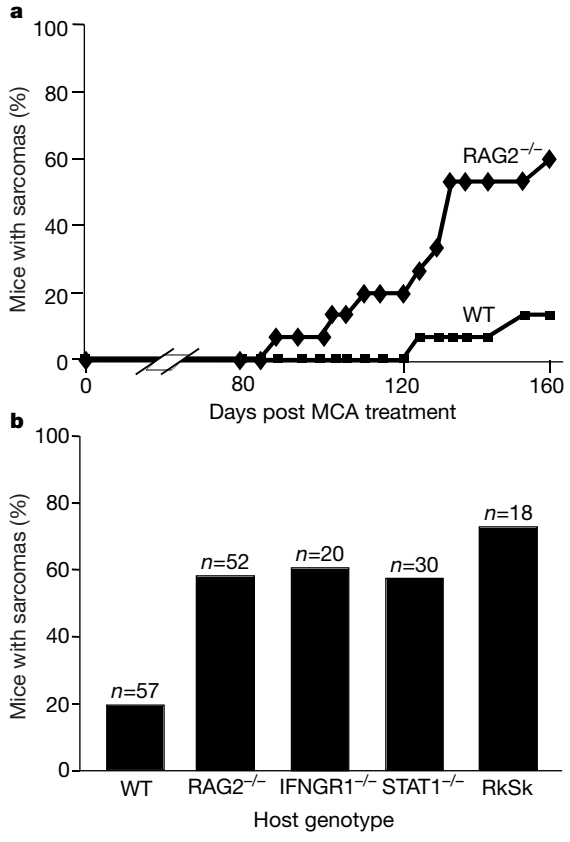

Figure 1 Lymphocyte-deficient mice are highly susceptible to MCA-induced tumour development. **a**, Fifteen female RAG2$^{-/-}$ mice (diamonds) and 15 syngeneic 129/SvEv wild-type (WT) mice (squares) were injected with a single subcutaneous dose of 100 μg MCA and observed for tumour development for 160 days. This dose and observation period were determined to be the most informative based on previous carcinogenesis experiments performed on the 129/SvEv strain of mice[9]. Data are represented as a percentage of each group of mice bearing tumours at least 9 mm in diameter as a function of time. **b**, Cumulative tumour formation at 160 days from three independent experiments involving female wild-type 129/SvEv mice ($n = 57$), RAG2$^{-/-}$ mice ($n = 52$), STAT1$^{-/-}$ mice ($n = 30$), IFNGR1$^{-/-}$ mice ($n = 20$) and RAG2$^{-/-}$ × STAT1$^{-/-}$ (RkSk) mice ($n = 18$) after injection of 100 μg MCA.

Given the comparable effects on tumour development of eliminating lymphocytes or STAT1-dependent IFNγ signalling, either individually or together, there is an extensive overlap between the two tumour-suppressor systems.

To determine whether these tumour-suppressor systems also function to prevent the formation of spontaneous tumours, we monitored tumour development in unmanipulated 129/SvEv wild-type mice, RAG2$^{-/-}$ mice and RkSk mice. As neither wild-type nor RAG2$^{-/-}$ mice showed outward signs of disease during the observation period, animals were killed and evaluated for neoplasia upon reaching a minimum age of 15 months. At necropsy, 9/11 wild-type mice (15–21 months) were free of neoplastic disease, one (16.1 months) developed an intestinal adenoma and one (19 months) displayed a harderian gland cystadenoma, but none had cancer (Fig. 2a and Supplementary Information). In contrast, 12/12 RAG2$^{-/-}$ mice of ages 15–16 months displayed neoplastic lesions in the intestinal tract and elsewhere (Fig. 2b and Supplementary Information). Half of these mice developed malignant neoplasias (three had caecal adenocarcinomas, one had an adenocarcinoma at the ileocaecal junction, one had an adenocarcinoma of the small intestine and one had a lung adenocarcinoma) that grew progressively when transplanted into naive RAG2$^{-/-}$ mice. Six of eleven RkSk mice developed overt mammary-gland carcinomas (one had two mammary adenocarcinomas and another carried distinct adenocarcinomas in the breast and caecum) that were detected well before the mice reached 15 months (Fig. 2c and Supplementary Information). This result is particularly striking given the extremely low frequency of spontaneous mammary tumours in wild-type mice or RAG2$^{-/-}$ mice of 129 origin (Fig. 2a, b) and the very late appearance (>20

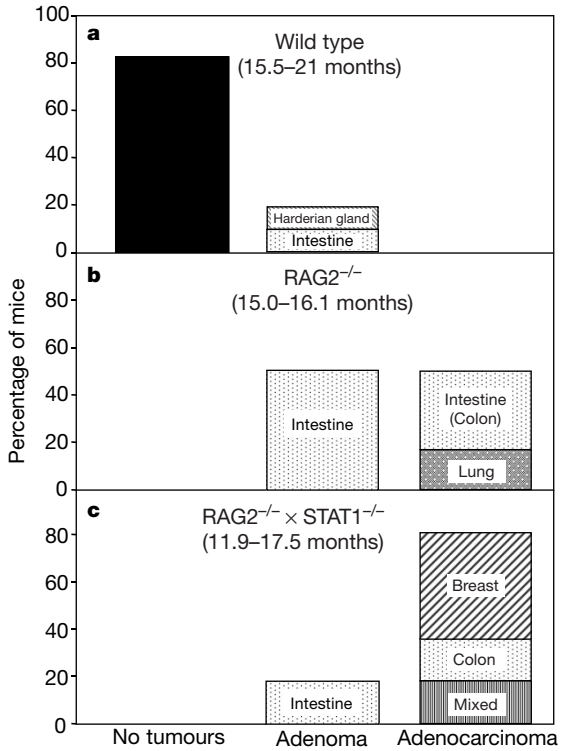

Figure 2 Increased development of spontaneous neoplastic disease in immunodeficient mice. Eleven wild-type 129/SvEv mice (**a**), 12 RAG2$^{-/-}$ mice (**b**) and 11 RkSk mice (**c**) were housed in a room that was free of specific pathogens including *Helicobacter*. Mice were killed when they appeared morbid, developed overt masses, or upon reaching an age of 15–21 months. Organs were examined for gross pathology, and histologic sections were analysed microscopically by three independent pathologists. Additional details of tumour formation and histologic sections from representative mice can be viewed as Supplementary Information.

months) of mammary tumours in mice that lack only the STAT1 gene (data not shown). Cells from mammary carcinomas grew progressively when transplanted into naive RkSk mice. The other five RkSk mice did not display palpable masses but at necropsy two had adenocarcinomas in the caecum, one had adenocarcinomas in the caecum and lung and the other two displayed intestinal adenomas. In total, 82% of the RkSk group developed spontaneous cancer. Thus, mice that lack lymphocytes, either alone or in combination with an IFNγ signalling deficit, are significantly more prone to spontaneous epithelial tumour development than their wild-type counterparts ($P < 0.01$ and 0.001, respectively). Moreover, because RkSk mice develop more spontaneous cancers than RAG2$^{-/-}$ mice ($P < 0.01$), the tumour-suppressor mechanisms mediated by lymphocytes and IFNγ/STAT1 must only partially overlap.

To assess whether the immune system influences the immunogenic phenotype of tumours formed during chemical carcinogenesis, the tumorigenicities of MCA-induced sarcomas from RAG2$^{-/-}$ and wild-type mice were compared using tumour transplantation approaches. When injected into RAG2$^{-/-}$ mice, both wild-type- and RAG2$^{-/-}$-derived sarcomas grew progressively with equivalent kinetics (Fig. 3a, b). When transplanted into naive syngeneic immunocompetent hosts, 17/17 different tumours from wild-type mice grew progressively (Fig. 3c). In contrast, 8/20 (40%) distinct tumours from MCA-treated RAG2$^{-/-}$ mice were rejected following transplantation into immunocompetent mice even when injected at high tumour cell inocula (10^6 cells per mouse) (Fig. 3d). The rejection of tumours derived from the immunodeficient hosts was not due to potential minor genetic differences between wild-type 129/SvEv and RAG2$^{-/-}$ 129/SvEv mice, as the tumours were rejected when transplanted into wild-type × RAG2$^{-/-}$ F1 mice. Moreover, we ruled out the possibility that the rejection of the RAG2$^{-/-}$ tumours was due to immune responses against retrovirus-related antigens as the tumours (1) did not produce infectious amphotropic or ecotropic retrovirus (plaque assays), (2) did not express retroviral gp70 messenger RNA (RT-PCR) and (3) did not express MuLV-related antigens (flow cytometry using G_{IX} MuLV antiserum[16]) (data not shown). Thus, tumours that arise in lymphocyte-deficient mice are more immunogenic than those that develop in the presence of an intact immune system. Taken together with our previous findings that IFNγ-unresponsive mice develop more tumours than wild-type mice and that IFNγ responsiveness of the tumour cell is essential for effective immune recognition[9], these results show that lymphocytes and the IFNγ/STAT1 signalling pathway collaborate with one another to shape the immunogenic phenotype of tumours that eventually form in immunocompetent hosts.

To determine the link between the lymphocyte-dependent and IFNγ/STAT1-dependent tumour-suppressor processes, we asked whether the highly tumorigenic phenotype of IFNγ-insensitive tumour cells could be eliminated by selectively expressing in them components of the MHC class I processing and presentation pathway that are known to be significantly upregulated by IFNγ. We studied two pathway components, TAP1 and the H-2K^b heavy chain, because the decreased or absent expression of these proteins is a mechanism that tumours use to escape immune detection[17]. This mechanism is also used by tumours that develop inactivating mutations in the genes that encode the proximal IFNγ receptor signalling proteins (ref. 9; V.S. & R.D.S., unpublished work). Our focus on TAP1 was also prompted by a previous report showing that expression of rat TAP1 in a mouse small-cell lung-carcinoma cell line decreased its tumorigenicity[18], although this study was complicated by the use of xenogeneic TAP1, which differs from the endogenous mouse protein at 80 positions[19].

We chose to use for these studies two distinct IFNγ-insensitive sarcomas (RAD.gR.28 and RAD.gR.30) derived from IFNGR1$^{-/-}$ 129/SvEv mice because we had (1) established previously that

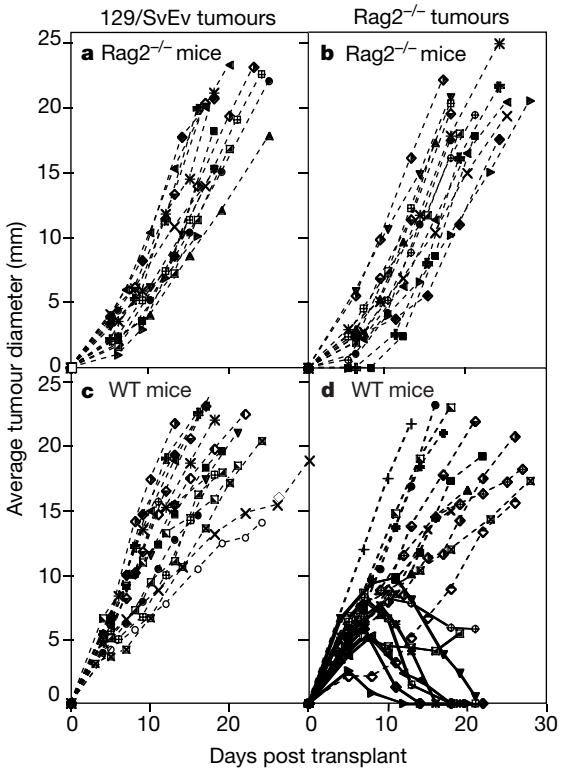

Figure 3 Increased immunogenicity of tumours derived from MCA-treated RAG2$^{-/-}$ mice. Immunodeficient RAG2$^{-/-}$ hosts were inoculated on day 0 with a dose of 10^5 tumour cells derived from wild-type 129/SvEv mice (**a**) or RAG2$^{-/-}$ mice (**b**). Tumour growth is plotted as mean tumour diameter of 3–5 mice inoculated with a distinct tumour. Groups of 5–8 immunocompetent 129/SvEv × RAG2$^{-/-}$ F1 mice were inoculated on day 0 with doses of 10^6 tumour cells derived from 17 individual 129/SvEv mice (**c**) or 20 individual RAG2$^{-/-}$ mice (**d**) and tumour growth monitored as above. In **d**, the dashed lines denote tumours that grew progressively, whereas solid lines represent tumours that were rejected.

sarcomas lacking either the IFNGR1 subunit or STAT1 display common biologic response deficits and enhanced *in vivo* growth and (2) defined, in great detail, the *in vivo* growth behaviour of IFNGR1$^{-/-}$ sarcomas before and after reconstitution of IFNγ sensitivity[9]. Both tumour cell lines, which express low but detectable amounts of TAP1 and H-2K^b protein (H-2K^b), were stably transfected with expression plasmids encoding the 129/SvEv haplotypes of TAP1 or H-2K^b, and clones were selected that expressed high protein levels comparable to those expressed in IFNγ-treated, IFNγ-responsive cells (see Supplementary Information). Parental RAD.gR28 cells (Fig. 4a), empty vector-transfected RAD.gR28.neo cells (Fig. 4b) and 2/2 clones of H-2K^b-transfected RAD.gR28.K^b cells (Fig. 4a) grew progressively in immunocompetent mice when injected at 10^6 cells per mouse. In contrast, TAP1-transfected RAD.gR28.TAP1 cells formed small subcutaneous masses that expanded for the first 5–10 days but then disappeared two weeks after inoculation (Fig. 4b). This effect was generalizable to 8/8 independent RAD.gR28.TAP1 clones (two representative clones are shown in Fig. 4b). In addition, the same result was obtained with 2/2 clones of a second IFNγ-insensitive tumour cell line (RAD.gR30) engineered for TAP overexpression (Fig. 4c). Rejection of TAP1-transfected IFNγ-insensitive tumour cells required the participation of both CD4$^+$ and CD8$^+$ T cells because rejection of the TAP1-reconstituted tumour cells occurred only in wild-type mice and not in lymphocyte-deficient RAG2$^{-/-}$ mice (Fig. 4d) and was abrogated in wild-type recipients depleted of either CD4$^+$ or CD8$^+$ T cells (Fig. 4e). We found that wild-type mice that rejected the TAP1-reconstituted IFNγ-insensitive tumour were immune to subsequent challenge with either the same reconstituted tumour cell line or the unreconstituted IFNγ-insensitive parental tumour cell line (Fig. 4f), a result that supports the concept that higher antigen expression is needed for the induction of an immune response to a tumour than is required for recognition of a tumour by a pre-sensitized host. Taken together, these results reveal that TAP1 represents at least one link between tumour suppression manifested by the IFNγ signalling pathway and lymphocytes.

In this study, we show that the immune response is essential to prevent the development of carcinogen-induced sarcomas and spontaneous epithelial tumours. We also show that the tumour-suppressor function of the immune system is critically dependent on the actions of IFNγ, which, at least in part, are directed at regulating tumour-cell immunogenicity. These studies thus provide experimental evidence that, in principle, supports the original concept of cancer immunosurveillance. However, as tumours that develop in the presence of an intact immune system are less immunogenic than those that develop in immunodeficient hosts, the actions of the immune system also paradoxically favour the eventual outgrowth of tumours that are more capable of escaping immune detection. Thus, tumours are imprinted by the immunologic environment in which they form. For this reason, 'cancer immunosurveillance' is not the best term to describe the process because the concept, in its original form, implied that the immune system is involved only at the initial stages of cellular transformation and plays a purely protective role[1,2]. Rather, we favour the use of the term 'cancer immunoediting' to describe better the protective and sculpting actions of the immune response on developing tumours that probably occur continuously during tumour development. We envisage the scope of this process to be very broad, with a potential

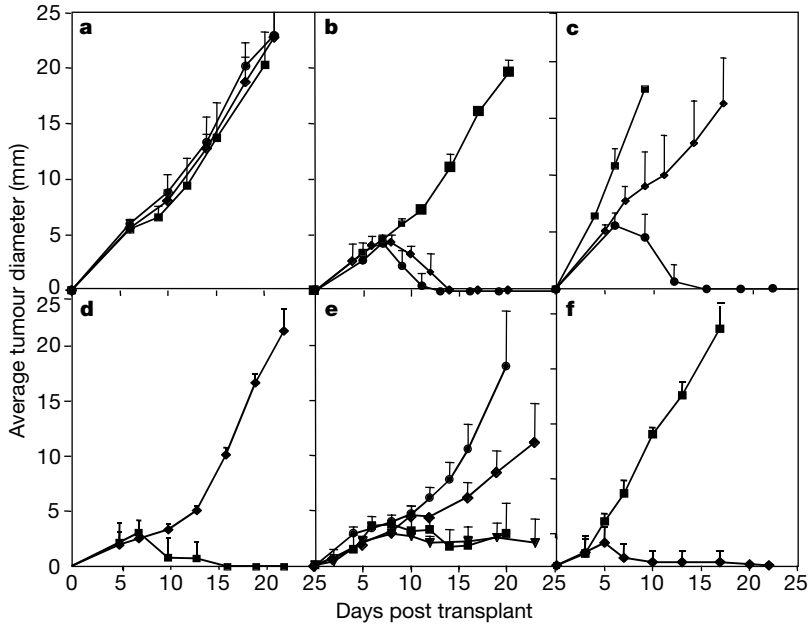

Figure 4 T-lymphocyte-dependent rejection of RAD.gR28.TAP1 cells by immunocompetent hosts. **a**, Wild-type mice were injected subcutaneously on day 0 with inocula of 10^6 RAD.gR28 (squares) cells or two representative H-2K^b-transfected RAD.gR28 clones denoted RAD.gR28.K^b.15 (diamonds) or RAD.gR28.K^b.30 (circles). **b**, One million mock-transfected RAD.gR28.neo cells (squares) or the same number of two representative TAP1-transfected RAD.gR28 clones denoted RAD.gR28.TAP1.8 (diamonds) or RAD.gR28.TAP1.16 (circles), were injected into syngeneic immunocompetent animals. **c**, 129/SvEv mice were injected with 10^6RAD.gR30 (squares), RAD.gR30.neo (diamonds) or RAD.gR30.TAP1 (circles) cells. Tumour diameter in the syngeneic hosts was monitored over time. **d**, RAD.gR28.TAP1 cells (10^6 cells per mouse) were injected into the flanks of wild-type mice (squares) or RAG2$^{-/-}$ mice (diamonds) and monitored for tumour growth. **e**, In a separate experiment RAD.gR28.TAP1 growth (10^6 cells per mouse) was compared after injection into wild-type 129/SvEv mice (squares) and wild-type mice depleted of CD8$^+$ T cells (circles) or wild-type mice (triangles) and wild-type mice depleted of CD4$^+$ T cells (diamonds). CD8$^+$ T cells were depleted by injecting mice intraperitoneally with 100 μg of purified YTS-169.4 anti-CD8 mAb seven days before tumour transplantation and once weekly thereafter. CD4$^+$ T cells were depleted by injection of 250 μg GK1.5 anti-CD4 mAb two days before tumour transplantation and every other day thereafter. Depletion of each T-cell subset was monitored by flow cytometry analysis on peripheral blood using FITC-labelled CD4 and CD8-specific mAbs directed against epitopes that were distinct from those recognized by the depleting antibodies. **f**, Growth of 10^6 parental RAD.gR28 tumour cells was assessed in naive 129/SvEv animals (squares) or 129/SvEv mice that had rejected on inoculum of 10^6 live RAD.gR28.TAP1 cells three weeks earlier (diamonds). Data are represented as average tumour diameter ± s.d. of 4–6 mice per group as a function of time.

to (1) promote complete elimination of some tumours, (2) generate a non-protective immune state to others, or (3) favour the development of immunologic anergy/tolerance/indifference. Future work is needed to define the molecular basis of the cancer immunoediting process. □

Methods

Mice

RAG2$^{-/-}$, IFNGR1$^{-/-}$ (ref. 20) and wild-type mice, all on a 129/SvEv background, were either purchased from Taconic Farms or bred in our specific pathogen-free animal facility. STAT1$^{-/-}$ mice, generated in our laboratory, were maintained on a pure 129/SvEv background[14]. For the generation of RAG2$^{-/-}$ × STAT1$^{-/-}$ mice, the status of the RAG2 locus was followed by assaying blood for CD3^{+} cells by FACS as previously described[21]. Genotyping of the STAT1 locus was performed by polymerase chain reaction as previously described[9].

MCA tumour induction and transplantation

Performed as previously described[9]. A new preparation of 3′-methylcholanthrene dissolved in peanut oil was used in each experiment. Progressively growing masses 9 mm and larger were scored as tumours and confirmed by histology. The threshold value of 9 mm was chosen because masses of this size invariably continued to increase in size. To eliminate the possibility that the rejection of tumours derived from RAG2$^{-/-}$ mice was due to minor antigenic differences between RAG2$^{-/-}$ mice and 129/SvEv mice, RAG2$^{-/-}$ or Taconic 129/SvEv tumours were transplanted into immunocompetent mice that were generated by mating male 129/SvEv mice (purchased from Taconic) to female RAG2$^{-/-}$ mice.

Determination of retrovirus expression in tumour cells

The feline S^{+}L^{-} focus assay (which tests for live amphotropic retrovirus) and the XC plaque assay (which tests for live B- and N-ecotropic murine retrovirus) were performed on supernatants from 12 different RAG2$^{-/-}$ tumour cultures by BioReliance. Eighteen different sarcomas from RAG2$^{-/-}$ mice and eleven different sarcomas derived from 129/SvEv wild-type animals were tested for reactivity to the G_{IX} rat antisera that reacts with several murine leukaemia virus (MuLV) proteins[16]. Primers within conserved regions of the gp70 gene were used to amplify a 335-base-pair band from the reverse-transcribed RNA of 16 different RAG2$^{-/-}$ tumours and 7 different wild-type tumours.

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1. Thomas, L. in *Cellular and Humoral Aspects of Hypersensitivity States* (ed. Lawrence, H. S.) 529–532 (Hoeber, New York, 1959).
2. Burnet, F. M. The concept of immunologic surveillance. *Prog. Exp. Tumor Res.* **13,** 1–27 (1970).
3. Stutman, O. Spontaneous tumors in nude mice: effect of the viable yellow gene. *Exp. Cell. Biol.* **47,** 129–135 (1979).
4. Stutman, O. Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice. *Science* **183,** 534–536 (1970).
5. Stutman, O. Chemical carcinogenesis in nude mice: comparison between nude mice from homozygous and heterozygous matings and effect of age and carcinogen dose. *J. Natl Cancer Inst.* **2,** 353–358 (1979).
6. Hunig, T. T-cell function and specificity in athymic mice. *Immunol. Today* **4,** 84–87 (1983).
7. Maleckar, J. R. & Sherman, L. A. The composition of the T cell receptor repertoire in nude mice. *J. Immunol.* **138,** 3873–3876 (1987).
8. Dighe, A. S., Richards, E., Old, L. J. & Schreiber, R. D. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFNγ receptors. *Immunity* **1,** 447–456 (1994).
9. Kaplan, D. H. *et al.* Demonstration of an IFNγ dependent tumor surveillance system in immunocompetent mice. *Proc. Natl Acad. Sci. USA* **95,** 7556–7561 (1998).
10. van den Broek, M. F. *et al.* Decreased tumor surveillance in perforin-deficient mice. *J. Exp. Med.* **184,** 1781–1790 (1996).
11. Smyth, M. J. *et al.* Differential tumor surveillance by natural killer (NK) and NKT cells. *J. Exp. Med.* **191,** 661–668 (2000).
12. Smyth, M. J. *et al.* Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. *J. Exp. Med.* **192,** 755–760 (2000).
13. Shinkai, Y. *et al.* RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68,** 855–867 (1992).
14. Meraz, M. A. *et al.* Targeted disruption of the STAT1 gene in mice reveals unexpected physiologic specificity in the Jak-STAT signaling pathway. *Cell* **84,** 431–442 (1996).
15. Durbin, J. E., Hackenmiller, R., Simon, M. C. & Levy, D. E. Targeted disruption of the mouse *Stat1* gene results in compromised innate immunity to viral infection. *Cell* **84,** 443–450 (1996).
16. Old, L. J. & Stockert, E. Immunogenetics of cell surface antigens of mouse leukemia. *Annu. Rev. Genet.* **17,** 127–160 (1977).
17. Marincola, F. M., Jaffee, E. M., Hicklin, D. J. & Ferrone, S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv. Immunol.* **74,** 181–273 (2000).
18. Alimonti, J. *et al.* TAP expression provides a general method for improving the recognition of malignant cells in vivo. *Nature Biotechnol.* **18,** 515–520 (2000).
19. Marusina, K., Iyer, M. & Monaco, J. J. Allelic variation in the mouse Tap-1 and Tap-2 transporter genes. *J. Immunol.* **158,** 5251–5256 (1997).
20. Huang, S. *et al.* Immune response in mice that lack the interferon-γ receptor. *Science* **259,** 1742–1745 (1993).
21. Dighe, A. S. *et al.* Tissue specific targeting of cytokine unresponsiveness in transgenic mice. *Immunity* **3,** 657–666 (1995).

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Somatic activation of the *K-ras* oncogene causes early onset lung cancer in mice

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About 30% of human tumours carry *ras* gene mutations[1,2]. Of the three genes in this family (composed of *K-ras*, *N-ras* and *H-ras*), *K-ras* is the most frequently mutated member in human tumours, including adenocarcinomas of the pancreas (~70–90% incidence), colon (~50%) and lung (~25–50%)[1–6]. To constuct mouse tumour models involving *K-ras*, we used a new gene targeting procedure to create mouse strains carrying oncogenic alleles of *K-ras* that can be activated only on a spontaneous recombination event in the whole animal. Here we show that mice carrying these mutations were highly predisposed to a range of tumour types, predominantly early onset lung cancer. This model was further characterized by examining the effects of germline mutations in the tumour suppressor gene *p53*, which is known to be mutated along with *K-ras* in human tumours. This approach has several advantages over traditional transgenic strategies, including that it more closely recapitulates spontaneous oncogene activation as seen in human cancers.

The effects of *ras* gene mutations have been studied in transgenic mice; however, most strains expressed *H-ras* or *N-ras* (reviewed in ref. 7). Traditional transgenic strategies direct expression of the oncogene in all cells of the target tissue and may lead to supra-physiological levels of expression. In an effort to construct a *ras*-based mouse tumour model that overcomes these limitations, we have used a variation of 'hit-and-run' gene targeting[8] to create new mouse strains harbouring latent, oncogenic alleles of *K-ras* capable of spontaneous activation *in vivo*.

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