

See discussions, stats, and author profiles for this publication at: <http://www.researchgate.net/publication/24254230>

# Host-Derived Interleukin-1 Is Important in Determining the Immunogenicity of 3-Methylcholantrene Tumor Cells

ARTICLE *in* THE JOURNAL OF IMMUNOLOGY · MAY 2009

Impact Factor: 4.92 · DOI: 10.4049/jimmunol.0803916 · Source: PubMed

CITATIONS

13

READS

20

12 AUTHORS, INCLUDING:



**Yakov Krelin**

Tel Aviv Sourasky Medical Center

22 PUBLICATIONS 1,497 CITATIONS

SEE PROFILE



**Angel Porgador**

Ben-Gurion University of the Negev

108 PUBLICATIONS 6,602 CITATIONS

SEE PROFILE



**Rachel G Lichtenstein**

Ben-Gurion University of the Negev

14 PUBLICATIONS 151 CITATIONS

SEE PROFILE



**Charles A Dinarello**

University of Colorado

1,080 PUBLICATIONS 93,514 CITATIONS

SEE PROFILE

# Host-Derived Interleukin-1 $\alpha$ Is Important in Determining the Immunogenicity of 3-Methylcholantrene Tumor Cells<sup>1</sup>

Moshe Elkabets,\* Yakov Krelin,\* Shahar Dotan,\* Adelheid Cerwenka,<sup>†</sup> Angel Porgador,\* Rachel G. Lichtenstein,<sup>\*§</sup> Malka R. White,\* Margot Zoller,<sup>†</sup> Yoichizo Iwakura,<sup>||</sup> Charles A. Dinarello,<sup>‡</sup> Elena Voronov,\* and Ron N. Apte<sup>2\*</sup>

Using IL-1/IL-1Ra knockout BALB/c mice, we showed that 3-methylcholantrene (3-MCA)-induced carcinogenesis is dependent on IL-1 $\beta$ -induced inflammatory responses. Patterns of local inflammation and tumorigenicity were similar in wild-type (WT) and IL-1 $\alpha$ <sup>-/-</sup> mice, while in IL-1 $\beta$ <sup>-/-</sup> mice, tumorigenicity was attenuated and in IL-1Ra<sup>-/-</sup> mice accentuated. 3-MCA-induced fibrosarcoma cell lines from WT mice developed into progressive tumors in WT mice, while surprisingly, lines from IL-1 $\alpha$ <sup>-/-</sup> mice formed tumors only in immunocompromized mice. 3-MCA-induced fibrosarcoma cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice, compared with lines from WT mice, manifested higher expression levels of “global” surface molecules related to Ag presentation and interactions with immune surveillance cells (MHC class I, B7.1, B7.2, L-selectin, and NKG2D ligands) and were eradicated mainly by CD4<sup>+</sup>- and CD8<sup>+</sup>-dependent T cell responses. Concomitantly, at the injection site of 3-MCA-induced fibrosarcoma cells derived from IL-1 $\alpha$ <sup>-/-</sup> mice, a leukocyte infiltrate, subsequently replaced by a scar-like tissue, was observed. Immune aberrations in NK cell maturation, antitumor specific immunity and killing capacity of effector cells were observed in IL-1 $\alpha$ <sup>-/-</sup> mice, in contrast to WT mice. Thus, we demonstrate in this study the significance of host-derived IL-1 $\alpha$  in cancer immunoediting, by affecting innate and specific immunosurveillance mechanisms. Overall, the results presented in this study, together with our previous studies, attest to differential involvement of IL-1 $\alpha$  and IL-1 $\beta$  in tumorigenesis; host-derived IL-1 $\beta$  mainly controls inflammation, while concomitantly, IL-1 $\alpha$  controls immunosurveillance of the arising malignant cells. Elucidation of the involvement of the IL-1 molecules in the malignant process will hopefully lead to the development of novel approaches for chemoprevention and immunotherapy. *The Journal of Immunology*, 2009, 182: 4874–4881.

**T**he association of chronic inflammation with the development of some types of cancer has been recognized and extensively investigated (reviewed in Ref. 1–7). In addition, tumorigenesis is affected by immunosurveillance mechanisms, which can determine the frequency of tumors and properties of the arising malignant cells (reviewed in Ref. 8–14). In vivo, in the milieu of developing tumors, inflammatory responses and im-

munosurveillance mechanisms coexist and their net interactions determine the fate of the malignant process.

We have assessed the role of IL-1, a most potent pleiotropic proinflammatory, immunostimulatory, and hemopoiesis-stimulating cytokine, on successive phases of the malignant process, i.e., tumorigenesis, tumor invasiveness and tumor-host interactions (reviewed in Ref. 1, 2, 15).

IL-1 consists of a family of agonistic and antagonistic molecules and receptors. IL-1 $\alpha$  and IL-1 $\beta$  are the major agonistic molecules of this family, while the IL-1 receptor antagonist (IL-1Ra) is a physiological inhibitor of preformed IL-1 (reviewed in Ref. 1, 2, 15–20). Although secreted IL-1 $\alpha$  and IL-1 $\beta$  bind to the IL-1RI signaling receptor and subsequently induce similar biological activities, in vivo they differ dramatically in their compartmentalization within the producing cell or the microenvironment (reviewed in Ref. 1, 2, 15, 17). This may account for the distinct effects of IL-1 $\alpha$  and IL-1 $\beta$  on the malignant process. Thus, both IL-1 molecules are generated as precursors that are further processed to generate the mature secreted molecule. IL-1 $\beta$  is only active in its mature secretable form and is thus a good inducer of extensive inflammatory responses. Indeed, we and others have shown that IL-1 $\beta$ , secreted by malignant cells or host cells in the tumor’s microenvironment, contributes to increased tumor invasiveness, tumor angiogenesis and tumor-mediated suppression (21–26). IL-1 $\alpha$ , in addition to its secreted mature form, is also active in cell-associated forms, i.e., the whole precursor molecule, the pro-piece, which is derived following cleavage of the precursor by calpain, and the functional membrane-associated form. Compared with IL-1 $\beta$ , IL-1 $\alpha$  is secreted in only a limited manner, especially by macrophages, and thus induces limited inflammatory responses and in its membrane-associated form it is immunostimulatory. Indeed,

\*The Shraga Segal Department of Microbiology and Immunology and Faculty of Health Sciences and The Cancer Research Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel; <sup>†</sup>German Cancer Research Center (DKFZ), Division of Innate Immunity (A.C.), and Department of Tumor Progression and Tumor Defense (M.Z.), Heidelberg, Germany; <sup>‡</sup>Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262; <sup>§</sup>Department of Biotechnology, Faculty of Engineering Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel; and <sup>||</sup>Institute of Medical Science, University of Tokyo, Tokyo, Japan

Received for publication November 26, 2008. Accepted for publication January 29, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup>R.N.A. was supported by the U.S.-Israel Binational Foundation (BSF), the Israel Ministry of Health Chief Scientist’s Office, and the German-Israeli DIP collaborative program (Deutsche-Israelische Projektkooperation). The Israel Science Foundation founded by the Israel Academy of Sciences and Humanities. National Institutes of Health Grants AI-15614, HL-68743, and CA-04 6934 (C.A. Dinarello). Cooperation Program in Cancer Research of the Deutsches Krebsforschungszentrum (DKFZ) and Israel’s Ministry of Science (MOS) (to A.P. and A.C.). R.N.A. is an incumbent of the Irving Isaac Sklar Chair in Endocrinology and Cancer. M.E. is supported by the ISEF Foundation and Nehemia-Lev-Zion scholarship.

<sup>2</sup>Address correspondence and reprint requests to Dr. Ron N. Apte, Department of Microbiology and Immunology, Faculty of Health Sciences and The Cancer Research Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel. E-mail address: rapte@bgu.ac.il

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/\$2.00

malignant fibrosarcoma cells which express cell-associated IL-1 $\alpha$ , which acts as a focused adjuvant, exhibit increased immunogenicity and induce regressing tumors in mice, through activation of anti-tumor cell immunity (24, 25, 27–31).

Using a model of 3-methylcholatrene (3-MCA)<sup>3</sup>-induced carcinogenesis in wild-type (WT) and IL-1/IL-1Ra deficient mice, we demonstrated the importance of IL-1 $\beta$ -induced inflammatory responses in tumorigenesis, while showing that host-derived IL-1 $\alpha$  is less significant in this process (21). IL-1Ra<sup>-/-</sup> mice exhibit the most rapid and strongest inflammatory responses, which persist throughout the process of tumorigenesis; enhanced tumor development is seen in all of these mice. The phenotype in WT and IL-1 $\alpha$ <sup>-/-</sup> mice was comparable; delayed inflammatory responses developed concomitantly with later tumor development in all mice, as compared with IL-1Ra<sup>-/-</sup> mice. In contrast, in IL-1 $\beta$ -deficient mice, there was only a very weak inflammatory response at the site of 3-MCA injection, not all of the mice developed tumors, and tumors developed later than in WT mice.

The immunogenic repertoire of the tumor cells, which arises during tumorigenesis, is shaped in the process of “tumor immunoeediting”, which consists of different phases of interaction between the malignant cells and host’s immunosurveillance mechanisms (elimination, equilibrium, and escape), as was initially characterized by the group of R.D. Schreiber (8, 9, 12–14, 32, 33). In immune intact mice, tumor immunoeediting culminates in the development of overt, progressively growing tumors, consisting of nonimmunogenic cells or cells that are able to induce tumor-mediated suppression. However, in mice deficient in cells or cytokines crucial for tumor immunosurveillance, immunogenic tumor cell variants are not properly eliminated during tumor progression and overt tumors consist of immunogenic cells that subsequently fail to develop into tumors when injected into secondary hosts. Components of the immune system that were shown to be involved in cancer immunoeediting include cells, i.e., lymphocytes and NKT cells, mediators, and surface molecules involved in tumor cell killing, such as perforin and TRAIL, or cytokines and molecules involved in their signaling, such as, IFN- $\gamma$ , IFN- $\gamma$ R1, IFNAR1, Stat1, IL-12, and IL-23 (reviewed in Ref. 8–12, 32, 33). In this study, we show that host-derived IL-1 $\alpha$  participates in cancer immunoeediting and we also demonstrate aberrations in immunosurveillance mechanisms in IL-1 $\alpha$ -deficient mice. Specific neutralization of IL-1 $\alpha$  and IL-1 $\beta$  production/function may thus prove instrumental to shift the balance between inflammation and immunosurveillance in the malignant process, in favor of reduced tumor invasiveness.

## Materials and Methods

### Mice

Female BALB/c mice were purchased from Harlan Sprague Dawley. The generation of IL-1 knockout (KO) mice, i.e., IL-1 $\alpha$ <sup>-/-</sup> and IL-1 $\beta$ <sup>-/-</sup> mice, were previously described (34). These mice were backcrossed to BALB/c mice for more than eight generations and are homozygous for the relevant mutation. IFN- $\gamma$  KO mice were purchased from The Jackson Laboratory. The IL-1 and IFN- $\gamma$  KO mice were bred and kept at the Animal Facilities of the Faculty of Health Sciences, Ben-Gurion University, under aseptic conditions. Mice were treated according to the Animal Care National Institutes of Health guidelines adapted by our Animal Committee.

### Induction of 3-MCA-induced tumors

Mice were injected s.c., into the right thigh with 3-MCA (200  $\mu$ g/mouse, Sigma-Aldrich) dissolved in olive oil. In this experimental system, local fibrosarcomas develop within 3–5 mo (21). Mice were examined twice a week for tumor development and size using a calibrated caliper. When

nonnecrotic tumors reached the diameter of 10–15 mm, mice were sacrificed and the tumor tissue was aseptically removed and the tumor was processed to establish cell lines by enzymatic digestion in trypsin (10 min at 37°C), as previously described by us (21).

### Cell lines

Isolated 3-MCA-induced fibrosarcoma cells from WT BALB/c mice and IL-1 $\alpha$ -deficient mice were cultured in DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin. All medium ingredients were purchased from Biological Industries. Cells were cultured at 37°C, 5% CO<sub>2</sub>, in a humidified incubator. YAC-1 (H-2a) cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin.

### Invasiveness of 3-MCA-induced cell lines

For determining tumorigenicity patterns of 3-MCA-induced cell lines from IL-1 $\alpha$ <sup>-/-</sup> and WT BALB/c mice, 1  $\times$  10<sup>5</sup> fibrosarcoma cells were injected i.f.p. into WT BALB/c mice. Tumor development was assessed twice a week using a caliper.

### Generation of CTLs

Mitomycin-C (Sigma-Aldrich)-treated 3-MCA-induced fibrosarcoma cell lines (100  $\mu$ g/10<sup>7</sup> cells for 1 h at 37°C) were injected i.p. (1  $\times$  10<sup>6</sup>/mouse); mice were subsequently given two booster injections with the same number of cells at 2-wk intervals. Splenocytes from immunized mice were subsequently harvested, challenged in culture, and further processed in experiments, as previously described (29).

### Adoptive transfer of spleen cells

Splenocytes from naive or immunized WT BALB/c and IL-1 $\alpha$ <sup>-/-</sup> mice were injected i.v. (15  $\times$  10<sup>6</sup>/mouse in 0.2 ml of PBS) into sublethally irradiated WT BALB/c mice (5 Gy of  $\gamma$ -Cobalt 60 per mouse; 600 rad), 24 h after irradiation. After an additional 24 h, fibrosarcoma cells were injected (1  $\times$  10<sup>7</sup>/mouse, i.f.p.) and tumor development was assessed.

### NK cell enrichment, RNA isolation, and real-time RT-PCR analysis of gene expression

Splenic NK cell were enriched using a commercial isolation kit for the negative selection of NK cells according to the manufacturer’s instructions (Miltenyi Biotec; Cat. no. 130-090-864). NK cell enrichment was verified by FACS analysis; ~85–90% of the recovered cells were CD3<sup>-</sup>DX5<sup>+</sup> CD122<sup>+</sup>.

Total RNA was isolated using the RNeasy Micro kit (Qiagen; Cat. no. No. 74004). cDNA was prepared using the Reverse Transcription System kit (Promega; Cat. no. 3500). All real-time PCR were performed in a 20  $\mu$ l mixture containing 1/20 concentration of 1 ng/ml cDNA preparation, 1 $\times$  Ready Mix SYBR Green Master Mix (Applied Biosystems; Cat. no. 4309155) and the relevant primers. Real-time quantifications were performed using the ABI 7500 System. The relative amount of transcript was calculated by the  $\Delta\Delta$ CT method, using the ABI 7500 System Software (version 1.2) and normalized to an endogenous reference gene, as indicated in the manufacturer’s instructions (Applied Biosystems).

The following primers were used: Endogenous  $\beta$ -actin: forward-GGTCTC AAACATGATCTGGG, reverse-GGGTCAGAAGGATTCTATG; Endogenous  $\beta$ -2 microglobulin: forward-TGGTGCTTGTCTCACTGACC, reverse-TATGTTTCGGCTCCCATCT; T-bet: forward-GTTCATTCCTGTCTT, reverse-CCTGTGTTGGTGAGCTT; GATA-3: forward-CTACCATA AAATGAATGGGCAGAA, reverse-CTCTCCTTGCTGCCGACAG; Granzyme: forward-GACCCTACATGGCCTTACTTT, reverse-CTCTGTGTTCTTGATGTTGTG; IFN- $\gamma$ : forward-TCAAGTGGCATAGATGTGGAAG, reverse-TGACGCTTATGTTGTTGCTGA 166bp; Perforin: forward-AAGG TAGCCAATTTTGCAGC, reverse-GGTTTTTGTACCAGGCGAAA. All primers were designed to cover exon boundaries and to enhance fragments 12–170 bp.

### Immunohistochemistry

Samples from the injection site of 3-MCA-induced fibrosarcoma cell lines were obtained on day 1, 2, and 14, fixed in 4% paraformaldehyde, dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Four-micron sections were stained with H&E, using established protocols (21).

For immunohistochemistry, tissue sections were processed as described previously (21). Abs were used as follows: purified rat anti-mouse Gr-1 (1/20; eBioscience), mouse mAbs anti-proliferating cell nuclear Ag (PCNA, 1/100; DakoCytomation), rat anti-human CD3 (Serotec, 1/100). The Vectastain Elite

<sup>3</sup> Abbreviations used in this paper: 3-MCA, 3-methylcholatrene; WT, wild type; KO, knockout; PCNA, anti-proliferating cell nuclear Ag.

ABC Peroxidase kit (Vector Laboratories) was used for secondary Ab application and detection (ApopTag plus peroxidase in situ apoptosis kit). Visualization was done by using AEC as a substrate (Zymed Laboratories). A pathologist examined the slides in a blind manner.

#### *In vivo depletion of lymphoid cell subpopulations*

In vivo depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was achieved via multiple i.p. injections of ascitic fluids containing the relevant mAbs, GK1.5 and YTS-169, respectively. Injection of Abs was performed on days -5, -3, and -1 before tumor cells injection ( $1 \times 10^5$  cells/mouse, i.f.p.) and twice a week thereafter. For in vivo NK depletion, mice were inoculated i.p. with 0.1 ml of anti-asialo GM1 rabbit serum (WAKO; Cat. no. 986-10001, 1/10 dilution in PBS). Anti-asialo GM1 was administered to mice on days -1 and +1 after tumor cell inoculation and then every 5 days. Depletion of specific lymphoid cells was verified in the spleen by FACS analysis.

#### *Cytolytic assay*

The cytolytic activity of NK cells against YAC-1 cells, which serve as a universal target for assaying NK-mediated lysis and of CTLs against the sensitizing tumor cells, was assessed in a 5-h <sup>35</sup>S-release assay (35). Percent of specific lysis was calculated as follows: % lysis = [(cpm experimental - cpm spontaneous release)/(cpm maximal release - cpm spontaneous release)]  $\times$  100. Spontaneous release was determined by incubation of the labeled target cells with medium. Maximal release was determined by solubilizing target cells in 0.1 M NaOH. In all presented experiments, the spontaneous release was <25% of the maximal release. Errors were usually <5% of the mean. Killing assays were performed in quadruplicates and shown is the mean and SD.

#### *Flow cytometry*

Spleens from WT BALB/c and IL-1 $\alpha$ <sup>-/-</sup> mice were removed and analyzed for NK cell markers. The mAbs that were used include FITC-labeled anti-CD3e (clone 145-2C11, BioLegend), PE-labeled anti-CD11b (clone M1/70, BioLegend), PE-labeled anti-B220 (clone RA3-6B2, BD Biosciences), allophycocyanin-labeled anti-CD49d (clone DX5, BioLegend), PE-labeled anti-CD122 (clone 5H4, eBioscience), PE-labeled anti-CD43 (L11, Miltenyi Biotec). Purified anti-CD16/CD32 Abs (BioLegend) were used to block the Fc receptor. Data files were acquired and analyzed using BD CellQuest 3.3 software. Fluorescence data were acquired using logarithmic amplification. Fluorescent axes in dot plots and histograms are in a logarithmic scale.

To detect surface markers on tumor cells, 3-MCA-induced tumor cell lines were harvested and blocked with PBS plus 0.5% FBS and purified anti-CD16/CD32 (BioLegend). Subsequently, they were stained for MHC class I-FITC conjugated mouse anti-H-2K<sup>d</sup> mAb (clone SF1-1.1 BD Pharmingen), FITC-labeled mouse anti-mouse I-A<sup>d</sup> (clone AF6-120.1, BD); B7.1- (allophycocyanin)-anti-mouse CD80 (clone 16-10A1, eBioscience) and B7.2- (allophycocyanin) anti-mouse CD86 (clone 16-10A1e, Bioscience); CD62L-PE-conjugated mAb anti-CD62L (clone MEL-14, BD Pharmingen). For detection of NKG2D ligands by flow cytometry, cell lines were stained with a mNKG2D-fusion protein (mNKG2D-FP), as previously described (36). A biotinylated donkey antihuman IgG antiserum (Jackson ImmunoResearch Laboratories) was used as a second step reagent, followed by staining with PE-conjugated streptavidin (Jackson ImmunoResearch Laboratories). The NCR1 fusion protein was generated as described (37) and staining of cell lines with it was visualized using secondary PE-conjugated goat-anti-human Abs (The Jackson Laboratory).

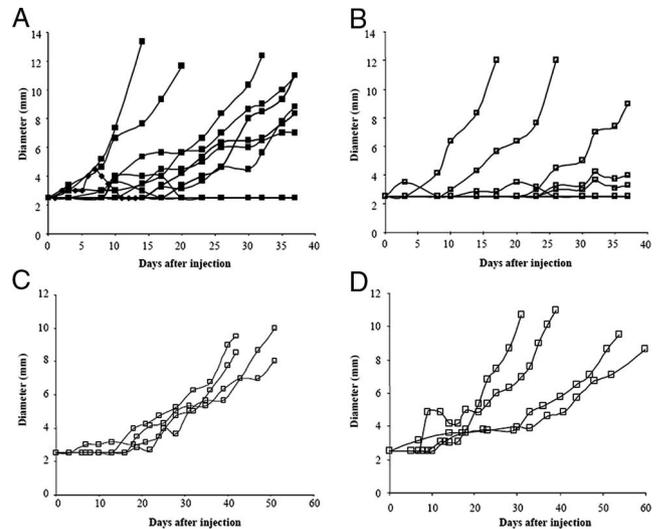
#### *Statistical analyses of the results*

Each experiment was repeated at least 3-5 times with similar patterns of responses. In vivo experiments of 3-MCA-induced tumorigenicity consisted of 10-20 mice in each experimental group, whereas in experiments on the invasiveness of 3-MCA-induced fibrosarcoma cell lines, experimental groups consisted of 5-10 mice. The data shown are from pooled or single representative experiments, as indicated, and are expressed as mean values  $\pm$  SEM. Significant differences in results were determined using the two-sided Student's *t* test; a *p* < 0.05 was considered significant.

## Results

### *3-MCA-induced fibrosarcoma cell lines derived from IL-1 $\alpha$ -deficient mice induce progressive tumors only in immunocompromized mice*

We have studied the effects of host-derived IL-1 on chemical carcinogenesis in response to 3-MCA. Deficiency of IL-1 $\alpha$  in the host did



**FIGURE 1.** Tumorigenicity patterns of 3-MCA in mice and reduced invasiveness of fibrosarcoma cell lines derived from IL-1 $\alpha$  KO mice. 3-MCA-induced fibrosarcoma cells derived from BALB/c ( $2 \times 10^5$ ) were injected i.f.p. into WT BALB/c mice and tumor development was assessed (■, 15 lines) (A). Tumorigenicity of 3-MCA-induced fibrosarcoma cells lines that were induced in IL-1 $\alpha$  KO mice (□, 16 lines) (B). Development of 3-MCA-induced fibrosarcoma cells derived from IL-1 $\alpha$  KO mice in IFN- $\gamma$  KO mice (C) or sublethally irradiated mice (5 Gy) (D). Each graph in represents patterns of invasiveness of one fibrosarcoma cell line (mean tumor diameter of 4-6 mice/experimental protocol). The invasive potential of each fibrosarcoma cell line under each experimental condition was repeated two to three times.

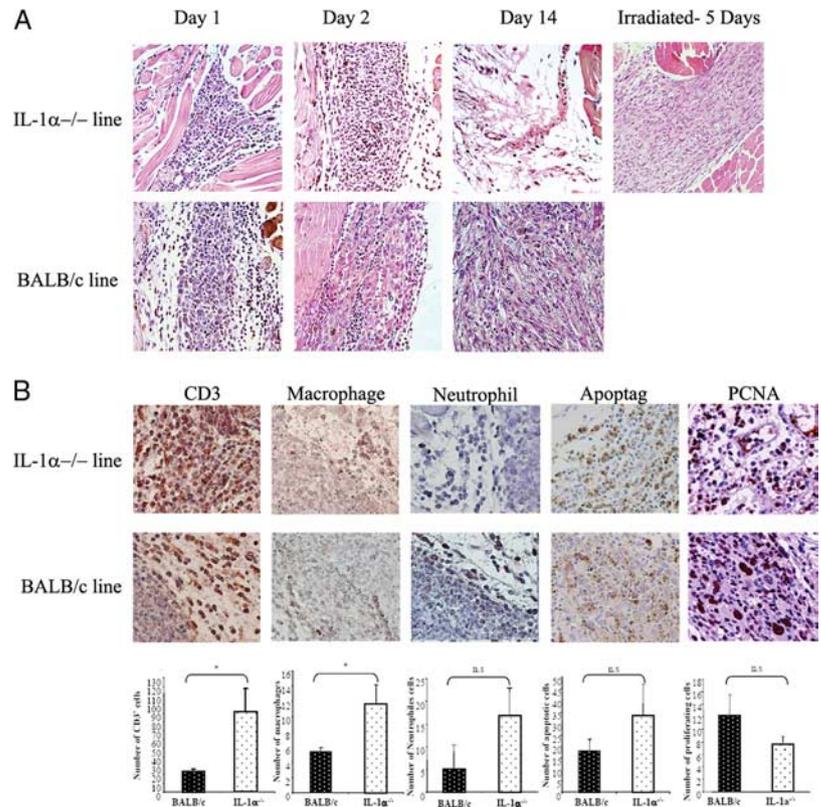
not significantly alter patterns of tumorigenesis in comparison to WT mice (21). When fibrosarcoma cell lines, obtained from 3-MCA-induced tumors in WT BALB/c mice, were injected into syngeneic hosts, ~81% of mice developed local progressive tumors (Fig. 1A). Surprisingly, when fibrosarcoma cell lines derived from IL-1 $\alpha$ <sup>-/-</sup> mice were injected into control mice, only ~15% of the lines developed into tumors, while most of the cell lines did not develop into tumors at all or started to develop and subsequently regressed, after reaching the diameter of 4-5 mm, roughly 30-40 days after tumor cell injection (Fig. 1B). These invasiveness patterns described above were consistent in most cell lines shown in Fig. 1; 12 of 15 lines from WT mice and 13 of 16 lines from IL-1 $\alpha$ <sup>-/-</sup> mice (Fig. 1). The vast majority of the 3-MCA cell lines derived from IL-1 $\alpha$ <sup>-/-</sup> mice did not grow in IL-1 $\alpha$ <sup>-/-</sup> mice, similarly to growth patterns in WT mice (data not shown). This phenotype of tumor cells derived from IL-1 $\alpha$ <sup>-/-</sup> mice was reproducible in ~30 cells derived from three 3-MCA-induced carcinogenesis experiments.

We next injected 3-MCA-induced cell lines, obtained from IL-1 $\alpha$ <sup>-/-</sup> mice, into different immunocompromized mice, such as sublethally irradiated mice (5 Gy) (Fig. 1C), IFN- $\gamma$ <sup>-/-</sup> mice (Fig. 1D), or CD-1 nude mice (data not shown). In these immunocompromized mice, all tumor cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice induced progressive tumors. This shows that tumor cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice are most likely immunogenic and did not lose their invasive potential. Similar progressive growth of 3-MCA-induced cell lines of WT origin was also observed in immunocompromized mice (data not shown).

### *Tumors from IL-1 $\alpha$ -deficient mice are eradicated by innate and specific immune mechanisms*

Histological analyses of the tissue response at the injection site of 3-MCA-induced fibrosarcoma cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice, which did not develop into tumors, have demonstrated early infiltration of leukocytes (CD3<sup>+</sup> T cells, macrophages, and

**FIGURE 2.** The tissue response at the site of injection of fibrosarcoma cell lines from IL-1 $\alpha$  KO mice. BALB/c mice were injected i.f.p. with tumor cells, as indicated in 1A. One, 2, and 14 days later, tissue samples from the site injection were collected, processed and stained with H&E, as described in *Materials and Methods* (magnification  $\times 20$ ). Also shown are immunohistochemistry stainings with CD3 Abs (lymphocytes), anti-Gr-1 (neutrophils), anti-F4/80 (macrophages), anti-PCNA Abs (replicating cells), and Apotag (apoptotic cells). In immunohistochemistry experiments shown are tissue sections from the site of tumor cell injection on day 2. Specific cell infiltration was quantified by calculation of stained cells under light microscopy, mean number of cells from different 4–6 fields was calculated, and statistical significance was analyzed. Statistical significance: \*\*,  $p < 0.01$  and \*,  $p < 0.05$  vs the appropriate controls in each panel. Shown are stainings from one experiment. The histological analyses were repeated twice and sections from three individual mice were checked at each experimental protocol.



neutrophils). Approximately 14 days postinjection, no malignant cells could be detected and a local scar-like tissue was observed. In sublethally irradiated mice, on day 5, 3-MCA-induced tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice induced a tumor mass, with scant infiltration of inflammatory cells. On the contrary, at the injection site of 3-MCA-induced cell lines from WT mice, there were fewer infiltrating cells, which remained in the periphery and, by day 14, a mass of tumor tissue was observed. Fig. 2B demonstrates the abundance of T cells, macrophages, and neutrophils in histological sections from the site of injection of 3-MCA-induced fibrosarcoma cells derived from IL-1 $\alpha$ <sup>-/-</sup> mice, in comparison to the infiltrate induced by similar tumor cells from WT mice (day 2). Shown are also immunohistochemical stainings of tissue sections with Apotag (Fig. 2B), demonstrating that cell apoptosis is more prominent in tissue sections from mice injected with 3-MCA-induced cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice, while proliferating cells (PCNA staining) were more abundant in tissue sections from mice injected with malignant cells from WT mice. The quantification of the immunohistochemical stainings is also shown.

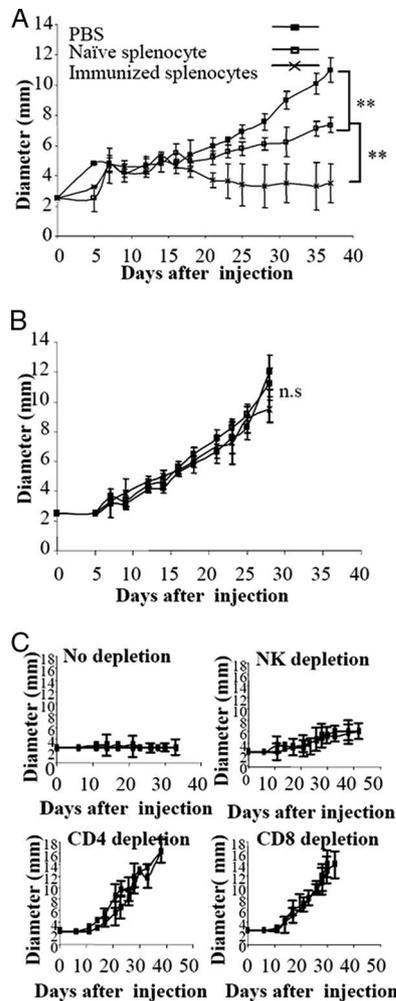
To further demonstrate the immunogenicity of tumor cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice, as compared with cell lines from WT mice, we used an adoptive transfer system. Thus, WT mice were sublethally irradiated and then transplanted with spleen cells from WT BALB/c mice (naive or immunized with either a cell line derived from IL-1 $\alpha$ <sup>-/-</sup> or WT mice). Twenty-four hours after cell transfer, mice were injected with the immunizing cell line and patterns of invasiveness were examined. As can be seen in (Fig. 3A), 3-MCA-induced tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice induced progressive growth in sublethally irradiated WT mice, while, as shown above, in untreated WT mice, no tumor development was observed. Injection of naive spleen cells from WT BALB/c mice into sublethally irradiated recipient mice reduced tumor growth, while tumor regression was observed following injection of spleen cells from WT mice immunized against the cell line from IL-1 $\alpha$ <sup>-/-</sup> mice. On the contrary, similar injection into sublethally irradiated mice, of either naive or immunized (against a cell

line-derived from WT mice) spleen cells, did not affect the progressive growth of cells from a fibrosarcoma cell line derived from WT BALB/c mice (Fig. 3B). These results indicate the immunogenicity of cells of the line from IL-1 $\alpha$ <sup>-/-</sup> mice and show that innate and specific immune effector cells affect their growth, while cell lines from WT mice were not affected by immune mechanisms and induced progressive tumors.

To further decipher the role of immune cells in the eradication of 3-MCA-induced cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice, depletion experiments were performed (Fig. 3C). Depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells induced progressive growth of 3-MCA-induced fibrosarcoma cells from IL-1 $\alpha$ <sup>-/-</sup> mice in WT BALB/c mice. NK depletion, with anti-asialo-GM1 Abs, induced initial growth of the malignant cells to form small tumors, ~3–4 mm in diameter, which did not further progress, or in some instances, even regressed. These results suggest that adaptive immunity mechanisms are involved in tumor rejection in IL-1 $\alpha$ <sup>-/-</sup> mice; NK cells have also some contribution to tumor cell rejection.

#### *Tumors from IL-1 $\alpha$ -deficient mice express molecules related to immunogenicity on their surface*

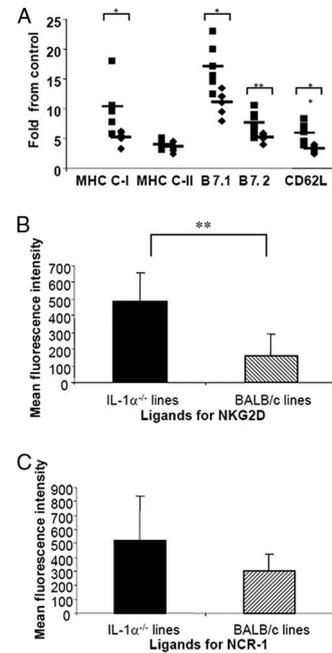
As tumor-associated Ags (TAAs) of fibrosarcoma cells are not characterized, we further assessed the expression of “global” markers of immunogenicity on 3-MCA-induced cell lines derived from IL-1 $\alpha$ <sup>-/-</sup> mice, compared with cell lines derived from WT BALB/c mice. For this purpose, cell surface molecules associated with Ag presentation and cell-to-cell interactions were analyzed by FACS analysis. The results shown in Fig. 4A indicate that tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice express more MHC class I, B7.1, B7.2, and CD62L (L-selectin) than counterpart cell lines derived from WT mice. Five to six cell lines of each phenotype were tested. No differences were found in the expression of MHC class II, FasL, CD1d, V-CAM, TRAIL, LFA-1, CD44, CD40, or ICAM-1 between the two types of fibrosarcoma cell lines. The NKG2D ligand that binds to the NK activator NKG2D receptor and potentiates the lytic machinery in



**FIGURE 3.** Innate and specific immunosurveillance cells limit the invasiveness of 3-MCA-induced fibrosarcoma cell lines derived from IL-1 $\alpha$  KO mice. Sublethally irradiated BALB/c mice were transplanted with naive splenocytes ( $\square$ ), immunized splenocytes ( $\times$ ) or splenocytes from PBS-treated mice ( $\blacksquare$ ), as described in *Materials and Methods*. Twenty hours later, mice were injected with 3-MCA-induced fibrosarcoma tumor cells derived from IL-1 $\alpha$  KO (A) or BALB/c (B) mice. Tumor size was measured by caliper every 2–3 days. Immunization was performed with the relevant cell line, as indicated in *Materials and Methods*. Shown are patterns of invasiveness of one cell line in one experiment (5–7 mice/group). Shown are the means and tumor diameter and the SD. Invasiveness of two cell lines from each phenotype under these experimental conditions was assessed. Shown are results of a representative experiment of two performed ( $n = 5$ –7 mice in each experimental treatment). Effect of lymphoid cell depletion on invasiveness of 3-MCA-induced fibrosarcoma cell lines derived from IL-1 $\alpha$  KO mice (C). Depletion of CD4 $^{+}$  and CD8 $^{+}$  T cells and NK cells in BALB/c mice was achieved following multiple i.p. injections of specific Abs. 3-MCA fibrosarcoma derived from IL-1 $\alpha$  KO mice were injected i.f.p. ( $1.5 \times 10^5$  cells/mouse) into depleted mice. Invasiveness from four cell lines from each phenotype ( $n = 5$ –7) for each experimental model, under these experimental conditions, were assessed in two independent experiments. Statistical significance: \*\*,  $p < 0.01$  and \*,  $p < 0.05$  vs the appropriate controls in each panel.

these cells was found at a higher frequency on the surface of 3-MCA tumor cells from IL-1 $\alpha^{-/-}$  mice (Fig. 4B). However, no significant difference in the expression of the NK inhibitory NCR1 ligand was observed when comparing these two types of cells (Fig. 4C).

Thus, 3-MCA-induced cell lines from IL-1 $\alpha^{-/-}$  mice are “enriched” in immunogenic markers, which probably contribute to



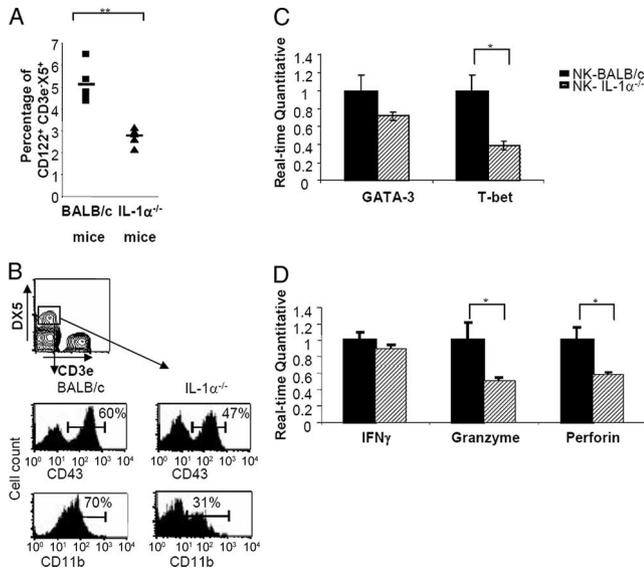
**FIGURE 4.** Surface markers on fibrosarcoma cell lines derived from IL-1 $\alpha$  KO mice. Expression of markers related to tumor immunogenicity on 3-MCA derived fibrosarcoma cell lines derived from IL-1 $\alpha$  KO compared with WT mice (A). Shown is the average fold increase in marker expression as assessed in six different 3-MCA-induced fibrosarcoma cell lines from IL-1 $\alpha$  KO ( $\blacksquare$ ) and WT BALB/c ( $\blacklozenge$ ) mice. All 3-MCA-induced fibrosarcoma cells were induced in one carcinogenesis experiment of five performed and analyzed. Surface marker expression was determined using stainings with specific Abs in FACS analyses and the index was calculated. Expression of NKG2DL (B) and NCR-1 (C) on the surface of five to six fibrosarcoma tumor cell lines from IL-1 $\alpha$  KO or BALB/c mice. MFI demonstrates surface protein expression levels per cell. Results are of one representative experiment of two performed. Shown is the mean of MFI and SD of five to six lines analyzed in a single experiment. Statistical significance: \*\*,  $p < 0.01$  and \*,  $p < 0.05$  vs the appropriate controls in each panel.

their rapid eradication in immune intact mice, compared with cell lines that originated in WT BALB/c mice.

#### *Reduced number/function of immunosurveillance cells in IL-1 $\alpha$ -deficient mice*

The results described herein suggest that, in IL-1 $\alpha^{-/-}$  mice, there is defect in tumor immunosurveillance during the process of carcinogenesis in IL-1 $\alpha^{-/-}$  mice and, as a result, immunogenic tumors appear, while tumor cells derived from WT mice are either nonimmunogenic or are able to induce tumor-mediated suppression. It was thus of interest to compare the number or function of innate and specific immune effector cells, which participate in tumor immunosurveillance, in WT and IL-1 $\alpha^{-/-}$  mice.

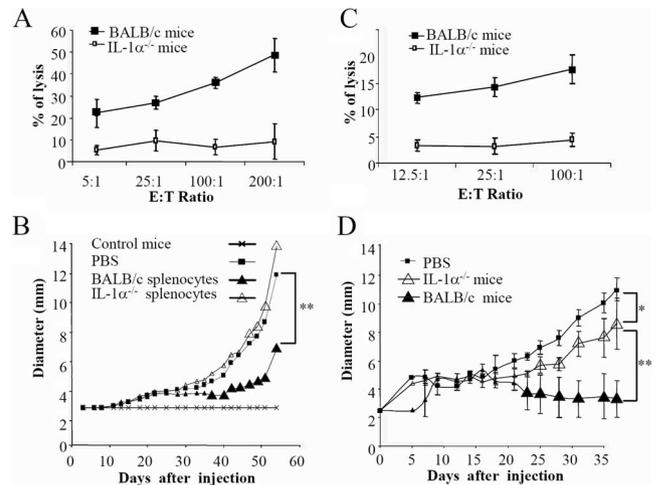
Initially, NK and NKT cells were compared in the spleen of naive BALB/c vs IL-1 $\alpha^{-/-}$  mice. Both types of spleen were of similar size and cell number (data not shown). In the spleen of IL-1 $\alpha^{-/-}$  mice, the percentage of CD3 $^{-}$ DX5 $^{+}$ CD122 $^{+}$  NK cells was  $\sim 50\%$  lower than that of WT mice (Fig. 5A). In addition, NK cells from IL-1 $\alpha^{-/-}$  mice, compared with such cells from WT mice, exhibited a less mature phenotype, as manifested by CD43 and CD11b expression (Fig. 5B). Furthermore, expression of the transcription factor, T-bet, which is important for the development and maturation of NK cells (and also Th1 cells) (38), was reduced in purified splenic NK cells from IL-1 $\alpha^{-/-}$  mice, compared with such cells from WT mice, while no significant difference in expression of GATA3 was observed (Fig. 5C). Transcription of genes related to molecules involved in killing, such



**FIGURE 5.** Characteristics of splenic NK cells derived from IL-1 $\alpha$  KO mice. Splenic NK cells from IL-1 $\alpha$  KO and WT BALB/c mice were obtained and purified as described in *Materials and Methods*. NK cells were analyzed for typical surface markers characterizing NK cell maturation, using specific Abs in FACS analyses. Demonstrated is the percentage of NK cells in the gated lymphocyte fraction of spleen cells (A) and maturation markers assessed on CD3<sup>-</sup>DX5<sup>+</sup>-gated cells (B). Expression of transcription factors related to NK cell maturation/function (C) and molecules related to NK cell function (D). Expression was assessed by RT-PCR, as described in *Materials and Methods* using RNA from purified NK cells. Results in A are from one representative experiment (mean values of five to ten individually tested mice of each strain) of three performed. C and D, patterns of NK cell maturation of molecules related to their function, (pooled purified NK cells from three mice of each strain). The results are from one representative experiment of three performed. Statistical significance: \*\*,  $p < 0.01$  and \*,  $p < 0.05$  vs the appropriate controls in each panel.

as granzyme and perforin, but not IFN- $\gamma$ , was also reduced in purified NK cells from IL-1 $\alpha$ <sup>-/-</sup> mice, compared with such cells from WT mice (Fig. 5D). Reduced killing capacity of Yac-1, target of NK cells, by spleen cells from IL-1 $\alpha$ <sup>-/-</sup> mice was observed at different E:T ratios, as compared with cells from WT BALB/c mice (Fig. 6A). Similar differences were also observed for the killing of IL-2-induced lymphokine-activated killer cells (LAKs) cells. In addition, NKT cells were detected in the liver, where they are most abundant, by staining with the  $\alpha$ -galactosylceramide tetramer and anti-CD3 Abs. Whereas in WT BALB/c mice, ~7% of mononuclear cells in the liver consist of NKT cells; in IL-1 $\alpha$ <sup>-/-</sup> mice, this population was ~10 times smaller (data not shown). The reduced numbers of NK and NKT cells were unique and no significant differences in the number of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells and B cells were observed between naive control and IL-1 $\alpha$ <sup>-/-</sup> mice (data not shown).

To further demonstrate the reduced activity of innate immune cells in the spleens of IL-1 $\alpha$ <sup>-/-</sup> mice, we used an adoptive transfer system. Thus, 3-MCA-induced tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice were injected into sublethally irradiated (5 Gy) WT mice, into which naive splenocytes from either WT BALB/c mice or IL-1 $\alpha$ <sup>-/-</sup> mice were injected. As was previously shown, 3-MCA-induced tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice grow progressively in sublethally irradiated WT mice; spleen cells from IL-1 $\alpha$ <sup>-/-</sup> mice did not significantly alter the progressive growth, whereas spleen cells from WT BALB/c mice inhibited tumor growth (Fig. 6B). Shown is also the inability of malignant cells to induce tumor cells in nontreated WT mice. These effects on the growth of tumor cells were observed in all three lines from IL-1 $\alpha$ <sup>-/-</sup> mice that were tested.



**FIGURE 6.** Lytic activity of splenic NK cells and anti-tumor-protective capacity of naive and immunized spleen cells from IL-1 $\alpha$  KO mice. Lytic capacity of splenic NK cells (A) were assessed in IL-1 $\alpha$  KO vs WT mice, as described in *Materials and Methods*. Antitumor protective capacity of naive spleen cells was assessed in an adoptive transfer experiment (B), as follows. Sublethally irradiated mice were transplanted with naive splenocytes from WT BALB/c or IL-1 $\alpha$  KO mice and 24 h later, the mice were injected with 3-MCA-induced fibrosarcoma cell lines derived from IL-1 $\alpha$  KO mice. IL-1 $\alpha$  KO and WT BALB/c mice were immunized with cells of an immunogenic 3-MCA-induced cell line derived from IL-1 $\alpha$  KO (mitomycin-C treated cells, three immunizations, as described in *Materials and Methods*). Specific lytic activity of splenic CTL was assessed (C) and the antitumor protective capacity of spleen cells was assessed in an adoptive transfer experiment, as indicated in *Materials and Methods*. In brief, sublethally irradiated mice were transplanted with immunized splenocytes from IL-1 $\alpha$  KO and WT mice. One day later, mice were injected with the immunizing 3-MCA-induced fibrosarcoma cell line (D). Shown are results of a representative experiment of three performed ( $n = 5-7$  mice in each experimental protocol). Statistical significance: \*\*,  $p < 0.01$  and \*,  $p < 0.05$  vs the appropriate controls in each panel.

In subsequent experiments, we assessed the killing and protective capacity of CTLs, which develop in IL-1 $\alpha$ <sup>-/-</sup> mice upon immunization, as compared with CTLs that develop in WT mice. Thus, IL-1 $\alpha$ <sup>-/-</sup> and WT BALB/c mice were immunized with a cell line derived from 3-MCA-treated IL-1 $\alpha$ <sup>-/-</sup> mice. Splenocytes from immunized mice were adoptively transferred into sublethally irradiated WT BALB/c mice that were subsequently challenged with the relevant tumor cells. Initially, we tested CTL lytic activity of splenic mononuclear cells from immunized IL-1 $\alpha$ <sup>-/-</sup> mice in comparison to WT BALB/c mice, showing higher lytic activity in spleen cells from WT mice, compared with spleen cells from IL-1 $\alpha$ <sup>-/-</sup> mice (Fig. 6C). We next assessed the protective capacity of spleen cells from immunized WT vs IL-1 $\alpha$ <sup>-/-</sup> mice in an adoptive transfer system, as described above. Spleen cells from immunized WT mice induced regression of tumors, while spleen cells from immunized IL-1 $\alpha$ <sup>-/-</sup> mice caused only limited growth inhibition (Fig. 6D). In total, these results show reduced numbers and function of innate and specific immunosurveillance cells in IL-1 $\alpha$ <sup>-/-</sup> mice, compared with cells from WT mice, indicating that micro-environmental IL-1 $\alpha$  is an important cytokine for immunosurveillance of malignant cells during the process of tumorigenesis.

## Discussion

The present study demonstrates that host-derived IL-1 $\alpha$  is instrumental in the process of immunoeediting during 3-MCA-induced carcinogenesis. The absence of IL-1 $\alpha$  possibly allows the survival of immunogenic variants of malignant cells that are otherwise eradicated

during tumor progression, in mice with a functioning immune system. Thus, 3-MCA-induced tumor cell lines derived from IL-1 $\alpha$ <sup>-/-</sup> mice are immunogenic and induce tumors only in immunocompromized mice, while 3-MCA-induced tumor cells from WT mice are either nonimmunogenic or induce tumor-mediated suppression and thus develop tumors also in immune intact mice.

We have previously shown that 3-MCA-induced carcinogenesis in WT and IL-1/IL-1Ra-deficient mice is controlled by IL-1 $\beta$ -induced inflammatory responses. Many studies, using the model of 3-MCA-induced carcinogenesis, have previously shown that immunosurveillance mechanisms determine the frequency of tumor development (reviewed in Ref. 9–14, 39)). The involvement of inflammation in 3-MCA carcinogenesis was previously described by the group of Blankenstein (39, 40) and was recently demonstrated in MyD88<sup>-/-</sup> mice, where a lower frequency of 3-MCA-induced fibrosarcomas, but no impairment in the immunogenicity of cells, was observed. Using 3-MCA carcinogenesis in IL-1 $\alpha$ <sup>-/-</sup> mice, we show that inflammation and immunosurveillance coexist *in vivo*; the delicate interaction between these processes, which affect the outcome of the malignant process, has to be established in individual tumor models, concerning the timing, nature, and magnitude of the relevant responses. Local spread or systemic inflammatory responses are usually protumorigenic, override manifestations of immunity, and even induce immunosuppression (like those observed in IL-1Ra<sup>-/-</sup> mice), while local weak to moderate inflammatory responses may potentiate innate immunity and further also activate adaptive antitumor cell immunity with the potential to limit tumor invasiveness.

TAA of 3-MCA-induced fibrosarcomas are not yet characterized and individual tumors are antigenically distinct. However, 3-MCA immunogenic cell lines, derived from IL-1 $\alpha$ <sup>-/-</sup> mice, as compared with similar cell lines from WT mice, express increased levels of surface “global” molecules, which are associated with Ag presentation and interactions of malignant cells with immunosurveillance cells. Expression of MHC class I molecules, costimulatory molecules (i.e., B7.1 and B7.2), and L-selectin is elevated in tumor cells derived from IL-1 $\alpha$ <sup>-/-</sup>, compared with WT mice. Thus, the immunogenic cells are enriched with MHC class I molecules that enable better presentation of tumor peptides to precursors of CTL, while B7.1 and B7.2 expression may facilitate direct presentation of tumor peptides by the malignant cells, which possibly synergizes with cross-presentation of tumor peptides by host-derived professional APCs. Enhanced expression of NKG2DL, the ligands of the NKG2D activator receptor on NK cells, on immunogenic tumor cells from IL-1 $\alpha$ <sup>-/-</sup> mice can potentially contribute to their direct killing by NK cells or to improved induction of antitumor immune responses, mediated by IFN- $\gamma$  secretion by NK cells. Previous experiments have described the role of NKG2D in immunosurveillance during tumorigenesis (41, 42).

In the experiment shown in Fig. 4, no differences in ICAM-1 expression were observed between immunogenic tumor cells from IL-1 $\alpha$ <sup>-/-</sup> vs WT mice. However, in another 3-MCA-induced carcinogenesis experiment, 11 of 12 lines derived from individual IL-1 $\alpha$ <sup>-/-</sup> mice expressed increased levels of ICAM-1, as compared with no cell lines of 7 tested of 3-MCA-induced cell lines from WT mice. Thus, in different 3-MCA carcinogenesis experiments in IL-1 $\alpha$ <sup>-/-</sup> mice, expression of different cell surface immunogenic molecules may differ however; impairment in immunosurveillance mechanisms in IL-1 $\alpha$ <sup>-/-</sup> mice is highly reproducible in all experiments. About thirty 3-MCA-induced fibrosarcoma cell lines from IL-1 $\alpha$ <sup>-/-</sup> and WT mice were tested.

Immunogenic tumor cell lines from IL-1 $\alpha$ <sup>-/-</sup> mice are rejected in immune intact mice by conventional innate cells, such as NK cells, and specific antitumor immune effector cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as evidenced in depletion experiments

(Fig. 3), histological analyses (Fig. 2), and adoptive transfer experiments (Fig. 3).

IL-1 is an important cytokine that participates in the development and activation of diverse innate and specific immune effector cells (reviewed in Ref. 1, 2, 15, 17). Accordingly, we have demonstrated some defects in immunosurveillance mechanisms in IL-1 $\alpha$ <sup>-/-</sup> mice that may affect tumor immunosurveillance. Thus, defects in maturation of NK cells in IL-1 $\alpha$ <sup>-/-</sup> mice were observed, concomitantly with reduced expression of T-bet, a transcription factor that controls NK cell maturation (43, 44). In addition, fewer liver NKT cells were detected in IL-1 $\alpha$ <sup>-/-</sup> mice than in WT mice. CD1d-restricted invariant NKT cells play a pivotal role in natural antitumor immune responses, mainly by activation of innate and adaptive immunosurveillance cells swift and potent cytokine secretion (45). Finally, in comparison to effector cells from WT mice, innate and specific antitumor effector cells from IL-1 $\alpha$ <sup>-/-</sup> mice, i.e., NK cells, LAK cells, and CTL, displayed a reduced killing capacity, which correlated with reduced expression of perforin and granzyme B in purified NK cells from IL-1 $\alpha$ <sup>-/-</sup> mice.

The unique membrane-associated form of IL-1 $\alpha$  is possibly of importance for its efficiency in activating antitumor immune responses, by acting as a focused adjuvant, through binding to IL-1RI, which are abundant on immunosurveillance cells, as has been demonstrated in our previous studies (24, 25, 27–31). Membrane IL-1 $\alpha$  may also be important in the effector phase of antitumor immunity, by enabling more avid binding between target tumor cells and immunosurveillance effector cells that leads to better killing. Alternatively, small amounts of IL-1 $\alpha$ , which is homeostatically expressed in cells, but not secreted, can be poured out from necrotizing cells at the site of carcinogen injection, and serve as a “danger signal” for mounting antitumor cells immunity (46). In contrast, IL-1 $\beta$  is only active as a secreted molecule, which is not accumulated in the producing cell; upon secretion, it possibly induces potent and widespread inflammatory responses that override, or even suppress, manifestations of adaptive immunity (21–25). In experimental models of autoimmune diseases, similar involvement of Th1 and Th17 cells, in induction and perpetuation of the pathogenic inflammatory responses, has been demonstrated. As Th1 cells are involved in induction of antitumor immunity, experiments are in progress to assess the involvement of Th17 and especially their interactions with molecules of the IL-1 family in response of the host against malignant cells.

Previous studies, using IL-1-deficient mice, have shown that IL-1 $\alpha$  is more dominant in cell-mediated immunity, while IL-1 $\beta$  is more involved in humoral immunity and induction of inflammation (47–49). However, IL-1 has not been described as a unique, indispensable factor for the development of immune responses in steady-state homeostasis. Also, 3-MCA-induced cell lines derived from IL-1 $\alpha$ <sup>-/-</sup> mice, do not induce progressive tumors in IL-1 $\alpha$ <sup>-/-</sup> recipients (data not shown), indicating that IL-1 $\alpha$ <sup>-/-</sup> mice are not extremely immunocompromized. The above-described deficiencies in immunosurveillance mechanisms in IL-1 $\alpha$ <sup>-/-</sup> compared with WT mice may be especially relevant under stress conditions, such as those in the process of chemical carcinogenesis. These immune deficiencies in IL-1 $\alpha$ <sup>-/-</sup> mice may be surmounted when the mice are confronted with a large inoculum of microbes, as multiple immune effector cells are then massively recruited from lymphoid organs to the periphery to confront the invader. However, these immune deficiencies possibly compromise the local and early eradication of immunogenic tumor cell variants, as they arise in the process of 3-MCA-induced carcinogenesis, by relatively low numbers of locally residing effector cells, without massive recruitment of immunosurveillance cells.

No impairment in immunoediting of tumor cells was observed in IL-1 $\beta$ -deficient mice, emphasizing the role of IL-1 $\alpha$ , rather than

IL-1 $\beta$ , in mounting antitumor immunity. However, due to the lack of expression of IL-1 $\beta$  by the malignant cells, they are unable to induce local inflammatory responses in the host and thus are defective in their invasive potential. However, injection of such tumor cells with an inflammation-inducing signal, such as LPS, results in progressive growth (data not shown). Further studies on the mechanisms of involvement of host-derived IL-1 $\alpha$  in tumor immunosurveillance will hopefully lead to better management of cancer by immune intervention protocols.

## Acknowledgements

We thank Dr. Lubov Gayvoronsky for help.

## Disclosures

The authors have no financial conflict of interest.

## References

- Apte, R. N., S. Dotan, M. Elkabets, M. R. White, E. Reich, Y. Carmi, X. Song, T. Dvorkin, Y. Krelin, and E. Voronov. 2006. The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions. *Cancer Metastasis Rev.* 25: 387–408.
- Apte, R. N., and E. Voronov. 2008. Is interleukin-1 a good or bad “guy” in tumor immunobiology and immunotherapy? *Immunol. Rev.* 222: 222–241.
- de Visser, K. E., and L. M. Coussens. 2006. The inflammatory tumor microenvironment and its impact on cancer development. *Contrib. Microbiol.* 13: 118–137.
- Lin, W. W., and M. Karin. 2007. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J. Clin. Invest.* 117: 1175–1183.
- Mantovani, A., P. Allavena, A. Sica, and F. Balkwill. 2008. Cancer-related inflammation. *Nature* 454: 436–444.
- Pikarsky, E., and Y. Ben-Neriah. 2006. NF- $\kappa$ B inhibition: a double-edged sword in cancer? *Eur. J. Cancer* 42: 779–784.
- Murdoch, C., M. Muthana, S. B. Coffelt, and C. E. Lewis. 2008. The role of myeloid cells in the promotion of tumor angiogenesis. *Nat. Rev. Cancer* 8: 618–631.
- Dunn, G. P., L. J. Old, and R. D. Schreiber. 2004. The immunobiology of cancer immunosurveillance and immunoeediting. *Immunity* 21: 137–148.
- Dunn, G. P., L. J. Old, and R. D. Schreiber. 2004. The three Es of cancer immunoeediting. *Annu. Rev. Immunol.* 22: 329–360.
- Swann, J. B., and M. J. Smyth. 2007. Immune surveillance of tumors. *J. Clin. Invest.* 117: 1137–1146.
- Ostrand-Rosenberg, S. 2008. Immune surveillance: a balance between protumor and antitumor immunity. *Curr. Opin. Genet. Dev.* 18: 11–18.
- Bui, J. D., and R. D. Schreiber. 2007. Cancer immunosurveillance, immunoeediting and inflammation: independent or interdependent processes? *Curr. Opin. Immunol.* 19: 203–208.
- Kim, R., M. Emi, and K. Tanabe. 2007. Cancer immunoeediting from immune surveillance to immune escape. *Immunology* 121: 1–14.
- Reiman, J. M., M. Kmiecik, M. H. Manjili, and K. L. Knutson. 2007. Tumor immunoeediting and immunosculpting pathways to cancer progression. *Semin. Cancer Biol.* 17: 275–287.
- Apte, R. N., Y. Krelin, X. Song, S. Dotan, E. Reich, M. Elkabets, Y. Carmi, T. Dvorkin, R. M. White, L. Gayvoronsky, et al. 2006. Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumor invasiveness and tumor-host interactions. *Eur. J. Cancer* 42: 751–759.
- Arend, W. P. 2002. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev.* 13: 323–340.
- Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87: 2095–2147.
- Mantovani, A., M. Muzio, P. Ghezzi, C. Colotta, and M. Introna. 1998. Regulation of inhibitory pathways of the interleukin-1 system. *Ann. NY Acad. Sci.* 840: 338–351.
- Sims, J. E. 2002. IL-1 and IL-18 receptors, and their extended family. *Curr. Opin. Immunol.* 14: 117–122.
- Stylianou, E., and J. Saklatvala. 1998. Interleukin-1. *Int. J. Biochem. Cell Biol.* 30: 1075–1079.
- Krelin, Y., E. Voronov, S. Dotan, M. Elkabets, E. Reich, M. Fogel, M. Huszar, Y. Iwakura, S. Segal, C. A. Dinarello, and R. N. Apte. 2007. Interleukin-1 $\beta$ -driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res.* 67: 1062–1071.
- Song, X., Y. Krelin, T. Dvorkin, O. Bjorkdahl, S. Segal, C. A. Dinarello, E. Voronov, and R. N. Apte. 2005. CD11b<sup>+</sup>/Gr-1<sup>+</sup> immature myeloid cells mediate suppression of T cells in mice bearing tumors of IL-1 $\beta$ -secreting cells. *J. Immunol.* 175: 8200–8208.
- Song, X., E. Voronov, T. Dvorkin, E. Fima, E. Cagnano, D. Benharroch, Y. Shendler, O. Bjorkdahl, S. Segal, C. A. Dinarello, and R. N. Apte. 2003. Differential effects of IL-1 $\alpha$  and IL-1 $\beta$  on tumorigenicity patterns and invasiveness. *J. Immunol.* 171: 6448–6456.
- Marhaba, R., I. Nazarenko, D. Knofler, E. Reich, E. Voronov, M. Vitacolonna, D. Hildebrand, E. Elter, R. N. Apte, and M. Zoller. 2008. Opposing effects of fibrosarcoma cell-derived IL-1 $\alpha$  and IL-1 $\beta$  on immune response induction. *Int. J. Cancer* 123: 134–145.
- Nazarenko, I., R. Marhaba, E. Reich, E. Voronov, M. Vitacolonna, D. Hildebrand, E. Elter, M. Rajasagi, R. N. Apte, and M. Zoller. 2008. Tumorigenicity of IL-1 $\alpha$ - and IL-1 $\beta$ -deficient fibrosarcoma cells. *Neoplasia* 10: 549–562.
- Bunt, S. K., P. Sinha, V. K. Clements, J. Leips, and S. Ostrand-Rosenberg. 2006. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J. Immunol.* 176: 284–290.
- Douvdevani, A., M. Huleihel, M. Zoller, S. Segal, and R. N. Apte. 1992. Reduced tumorigenicity of fibrosarcomas which constitutively generate IL-1 $\alpha$  either spontaneously or following IL-1 $\alpha$  gene transfer. *Int. J. Cancer* 51: 822–830.
- Voronov, E., Y. Weinstein, D. Benharroch, E. Cagnano, R. Ofir, M. Dobkin, R. M. White, M. Zoller, V. Barak, S. Segal, and R. N. Apte. 1999. Antitumor and immunotherapeutic effects of activated invasive T lymphoma cells that display short-term interleukin 1 $\alpha$  expression. *Cancer Res.* 59: 1029–1035.
- Zoller, M., A. Douvdevani, S. Segal, and R. N. Apte. 1992. Interleukin-1 production by transformed fibroblasts, II: influence on antigen presentation and T-cell-mediated anti-tumor response. *Int. J. Cancer* 50: 450–457.
- Zoller, M., A. Douvdevani, S. Segal, and R. N. Apte. 1992. Interleukin-1 produced by tumorigenic fibroblasts influences tumor rejection. *Int. J. Cancer* 50: 443–449.
- Dvorkin, T., X. Song, S. Argov, R. M. White, M. Zoller, S. Segal, C. A. Dinarello, E. Voronov, and R. N. Apte. 2006. Immune phenomena involved in the in vivo regression of fibrosarcoma cells expressing cell-associated IL-1 $\alpha$ . *J. Leukocyte Biol.* 80: 96–106.
- Dunn, G. P., A. T. Bruce, H. Ikeda, L. J. Old, and R. D. Schreiber. 2002. Cancer immunoeediting: from immunosurveillance to tumor escape. *Nat. Immunol.* 3: 991–998.
- Smyth, M. J., G. P. Dunn, and R. D. Schreiber. 2006. Cancer immunosurveillance and immunoeediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv. Immunol.* 90: 1–50.
- Horai, R., M. Asano, K. Sudo, H. Kanuka, M. Suzuki, M. Nishihara, M. Takahashi, and Y. Iwakura. 1998. Production of mice deficient in genes for interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-1 $\alpha/\beta$ , and IL-1 receptor antagonist shows that IL-1 $\beta$  is crucial in turpentine-induced fever development and glucocorticoid secretion. *J. Exp. Med.* 187: 1463–1475.
- Mandelboim, O., N. Lieberman, M. Lev, L. Paul, T. I. Arnon, Y. Bushkin, D. M. Davis, J. L. Strominger, J. W. Yewdell, and A. Porgador. 2001. Recognition of haemagglutinins on virus-infected cells by NKP46 activates lysis by human NK cells. *Nature* 409: 1055–1060.
- Cerwenka, A., A. B. Bakker, T. McClanahan, J. Wagner, J. Wu, J. H. Phillips, and L. L. Lanier. 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 12: 721–727.
- Gazit, R., R. Gruda, M. Elboim, T. I. Arnon, G. Katz, H. Achdout, J. Hanna, U. Qimron, G. Landau, E. Greenbaum, et al. 2006. Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nat. Immunol.* 7: 517–523.
- Townsend, M. J., A. S. Weinmann, J. L. Matsuda, R. Salomon, P. J. Farnham, C. A. Biron, L. Gapin, and L. H. Glimcher. 2004. T-bet regulates the terminal maturation and homeostasis of NK and V $\alpha$ 14i NKT cells. *Immunity* 20: 477–494.
- Blankenstein, T., and Z. Qin. 2003. Chemical carcinogens as foreign bodies and some pitfalls regarding cancer immune surveillance. *Adv. Cancer Res.* 90: 179–207.
- Qin, Z., H. J. Kim, J. Hemme, and T. Blankenstein. 2002. Inhibition of methylcholanthrene-induced carcinogenesis by an interferon  $\gamma$  receptor-dependent foreign body reaction. *J. Exp. Med.* 195: 1479–1490.
- Guerra, N., Y. X. Tan, N. T. Joncker, A. Choy, F. Gallardo, N. Xiong, S. Knoblaugh, D. Cado, N. R. Greenberg, and D. H. Raulet. 2008. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity* 28: 571–580.
- Unni, A. M., T. Bondar, and R. Medzhitov. 2008. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. *Proc. Natl. Acad. Sci. USA* 105: 1686–1691.
- Werneck, M. B., G. Lugo-Villarino, E. S. Hwang, H. Cantor, and L. H. Glimcher. 2008. T-bet plays a key role in NK-mediated control of melanoma metastatic disease. *J. Immunol.* 180: 8004–8010.
- Robbins, S. H., M. S. Tessler, L. Van Kaer, and L. Brossay. 2005. Direct effects of T-bet and MHC class I expression, but not STAT1, on peripheral NK cell maturation. *Eur. J. Immunol.* 35: 757–765.
- Berzofsky, J. A., and M. Terabe. 2008. NKT cells in tumor immunity: opposing subsets define a new immunoregulatory axis. *J. Immunol.* 180: 3627–3635.
- Chen, C. J., H. Kono, D. Golenbock, G. Reed, S. Akira, and K. L. Rock. 2007. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.* 13: 851–856.
- Nakae, S., M. Asano, R. Horai, and Y. Iwakura. 2001. Interleukin-1 $\beta$ , but not interleukin-1 $\alpha$ , is required for T-cell-dependent antibody production. *Immunology* 104: 402–409.
- Nakae, S., C. Naruse-Nakajima, K. Sudo, R. Horai, M. Asano, and Y. Iwakura. 2001. IL-1 $\alpha$ , but not IL-1 $\beta$ , is required for contact-allergen-specific T cell activation during the sensitization phase in contact hypersensitivity. *Int. Immunol.* 13: 1471–1478.
- Nambu, A., S. Nakae, and Y. Iwakura. 2006. IL-1 $\beta$ , but not IL-1 $\alpha$ , is required for antigen-specific T cell activation and the induction of local inflammation in the delayed-type hypersensitivity responses. *Int. Immunol.* 18: 701–712.