Interleukin 12–mediated Prevention of Spontaneous Mammary Adenocarcinomas in Two Lines of Her-2/*neu* Transgenic Mice

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Summary

The ability of interleukin (IL)-12 to prevent tumors when administered to individuals with a genetic risk of cancer was studied in two lines of transgenic mice expressing rat HER-2/*neu* oncogene in the mammary gland. Female BALB/c (H-2^d) mice carrying the activated HER-2/*neu* oncogene show no morphological abnormalities of the mammary gland until 3 wk of age. They then progress through atypical hyperplasia to in situ lobular carcinoma and at 33 wk of age all 10 mammary glands display invasive carcinomas. Adult FVB mice (H-2^q) carrying the HER-2/*neu* protooncogene develop mammary carcinomas with a longer latency (38-49 wk) and a lower multiplicity (mean of 2.6 tumors/mice). Treatment with IL-12 (5 daily intraperitoneal injections, 1 wk on, 3 wk off; the first course with 50 ng IL-12/day, the second with 100 ng IL-12/day) begun at 2 wk of age in BALB/c mice and at 21 wk of age in FVB mice markedly delayed tumor onset and reduced tumor multiplicity. Analogous results were obtained in immunocompetent and permanently CD8$^+$ T lymphocyte–depleted mice. In both transgenic lines, tumor inhibition was associated with mammary infiltration of reactive cells, production of cytokines and inducible nitric oxide synthase, and reduction in microvessel number, in combination with a high degree of hemorrhagic necrosis.

Key words: interleukin 12 • adenocarcinomas • tumor prevention • angiogenesis • Her-2/*neu*

Current genetic studies are leading to the identification of gene mutations that predispose to cancer. They thus hold out the hope that not-yet affected patients or "unpatients" with a defined genetic prognosis can be detected (1). This, indeed, is already a reality in breast cancer, one of the most common malignancies in women.

With the paucity of effective preventive options, probing of the human genome is raising new and dramatic ethical, psychological, and cultural issues (1). On the other hand, identification of a mutated gene and its altered or amplified products is providing a new chance of undertaking immunologic maneuvers against oncogene products (2) in an unprecedented setting, where they may be able to prevent the onset or inhibit the initial growth of tumors in healthy individuals with a high risk of cancer.

This paper offers evidence of the efficacy of administration of mouse recombinant IL-12 (rIL-12) in counteracting the occurrence and progression of spontaneous mammary carcinomas in the females of two lines of inbred mice transgenic for the rat HER-2/*neu* oncogene under the transcriptional control of mouse mammary tumor virus (MMTV)[1] LTR (3). BALB–NeuT female mice are from a new line of transgenic BALB/c mice carrying the activated HER-2/*neu* oncogene (4) and presenting high mammary

[1]*Abbreviations used in this paper:* iNOS, inducible NO synthase; IP-10, IFN-γ–inducible protein 10; NO, nitric oxide; MIG, monokine induced by γ-IFN; MMTV, mouse mammary tumor virus; MSA, mouse serum albumin; VCAM, vascular cell adhesion molecule.

tumor multiplicity and relatively fast tumor growth (5). FVB–NeuN females carry the HER-2/*neu* protooncogene (6) and display lower multiplicity and longer latency. Prolonged administration of low doses of rIL-12 delayed tumor onset and reduced tumor multiplicity in both lines. Analogous results were obtained in BALB–NeuT mice permanently depleted of $CD8^+$ T lymphocytes.

Tumor inhibition in both lines was associated with deficient peri- and intratumoral angiogenesis, infiltration of reactive cells, production of proinflammatory cytokines, and inducible nitric oxide synthase (iNOS) activation. Vascular damage resulted in a high degree of hemorrhagic necrosis of established tumor masses.

Materials and Methods

Mice. A transgenic CD1 random-bred breeder male mouse (no. 1330) carrying the mutated rat HER-2/*neu* oncogene driven by the MMTV promoter (Tg-NeuT, provided by Dr. L. Clerici, Euratom, Ispra, Italy; reference 5) was mated with BALB/c females (H-2^d; Charles River, Calco, Italy). The progeny was screened for the transgene by PCR. Transgene-carrying males were backcrossed with BALB/c females for 12 generations and HER-2/*neu* $^+$ BALB/c mice (BALB–NeuT) were used in these experiments. Parental FVB–NeuN N#202 transgenic mice (6) carrying the rat HER-2/*neu* protooncogene driven by the MMTV promoter on the H-2^q FVB inbred background were provided by Dr. W.J. Muller (McMaster University, Hamilton, Ontario, Canada) and bred in our animal facilities. Females of both transgenic lines show a MMTV-driven overexpression of the transgene in the mammary gland and a definite tumor growth involving the mammary gland epithelium (5–7). Individually tagged virgin females were used in this study. Starting at the age of 5 wk, their mammary glands were inspected once a week, and masses were measured with calipers in the two perpendicular diameters (8). Progressively growing masses >3 mm mean diameter were regarded as tumors. BALB–NeuT mice were killed at wk 33 when these masses were evident in all 10 mammary glands. FVB–NeuN mice were killed when a mammary mass exceeded 2 cm mean diameter, and surviving mice were killed at 61 wk. All mice were evaluated histologically for mammary tumor development and toxicity related to IL-12 administration.

IL-12 Administration. rIL-12 (Genetics Institute, Cambridge, MA) in HBSS supplemented with 0.01% mouse serum albumin (MSA; Sigma Chemical Co., St. Louis, MO) was administered intraperitoneally. MSA control mice received similar injections of MSA only.

Depletion of $CD8^+$ Lymphocytes. After thymectomy, a few BALB–NeuT neonatal mice were thymectomized and received 200 μg of purified anti-$CD8^+$ cell mAb (53.6.72 hybridoma, anti-Lyt2; American Type Culture Collection, Rockville, MD) injec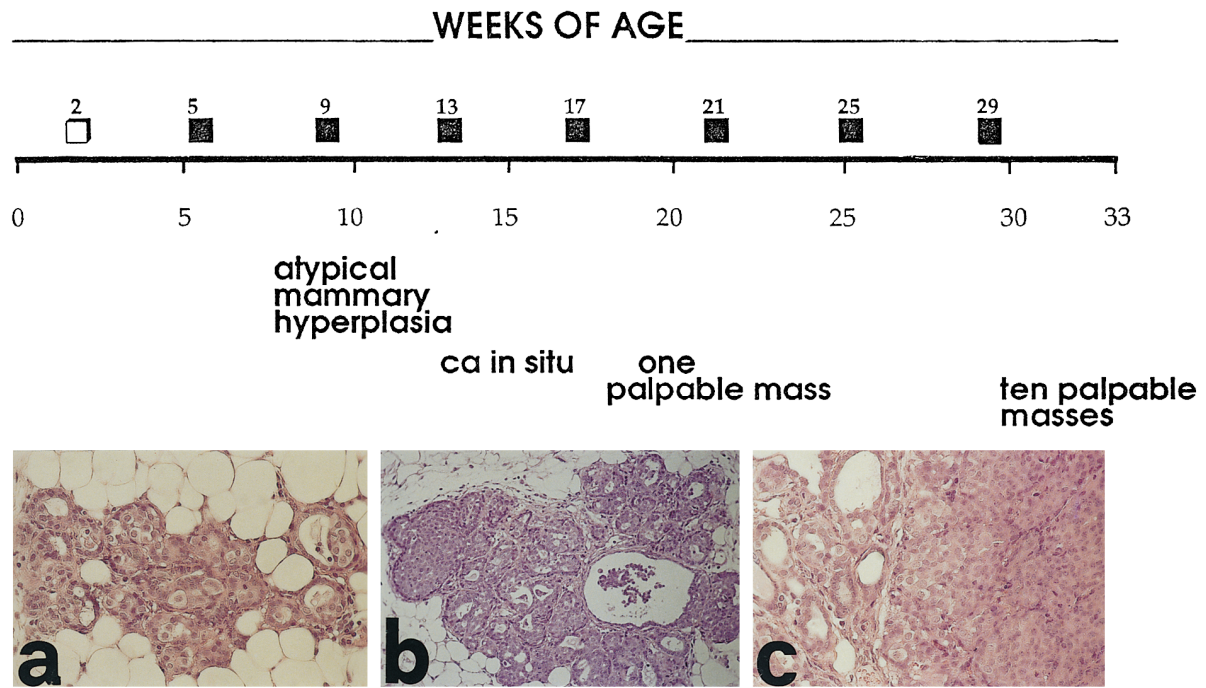ted intraperitoneally 2 d later. Complete removal of thymus lobes and effective $CD8^+$ T lymphocyte depletion was checked at 33 wk. Immunofluorescence of lymph node cells was performed by staining with FITC-conjugated rat anti mouse CD3 (clone 145-2C11), CD4 (clone RM4-4), or CD8 (clone 53-6.72) mAbs (all from PharMingen, San Diego, CA). Cytofluorometry showed a persistent reduction and depletion of $CD8^+$ lymphocytes (<4%) compared with untreated mice.

Morphologic Analysis. Groups of two or three BALB–NeuT mice were killed at wk 2 and 3 and then every other week until wk 33; similar groups of FVB–NeuN were killed every 4 wk from wk 5 to 61. For histologic evaluation, tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin or Giemsa. For immunohistochemistry, acetone-fixed cryostat sections were incubated for 30 min with anti-CD4, anti-CD8a (all from Sera-Lab, Crawley Down, Sussex, UK), anti-Mac-1 (anti CD11b/CD18), anti–Mac-3, and anti-Ia (all from Boehringer Mannheim, Milan, Italy), antipolymorphonuclear leukocytes (RB6-8C5; provided by Dr. R.L. Coffman, DNAX Inc., Palo Alto, CA), antiendothelial cells (mEC-13.324; reference 9), anti–IL-1α (Genzyme Corp., Cambridge, MA), anti–TNF-α (Immuno Kontact, Frankfurt, Germany), anti–IFN-γ (10), anti–IL-6 (PharMingen), and anti-iNOS (Transduction Laboratories, Lexington, KY) antibodies. After washing, the cryostat sections were overlaid with biotinylated goat anti–rat, anti–hamster, and anti–rabbit or horse anti–goat Igs (Vector Labs., Burlingame, CA) for 30 min. Unbound Ig was removed by washing and the slides were incubated with avidin–biotin complex (ABC)/alkaline phosphatase (AP) (Dako, Glostrup, Denmark). Quantitative studies of immunohistochemically stained sections were performed independently by three pathologists in a blind fashion. From mice with multiple tumors, 2 or more samples (1/tumor growth area) and 10 randomly chosen fields in each sample were evaluated for each point determination. For microvessel and cell counts, individual microvessels and cells were counted under a microscope ×400 field (×40 objective and ×10 ocular lens; 0.180 mm² per field). The expression of adhesion molecules, cytokines, and mediators was defined as absent ($-$) or scarcely ($+/-$), moderately ($+$), or frequently ($++$) present on cryostat sections tested with the corresponding antibodies.
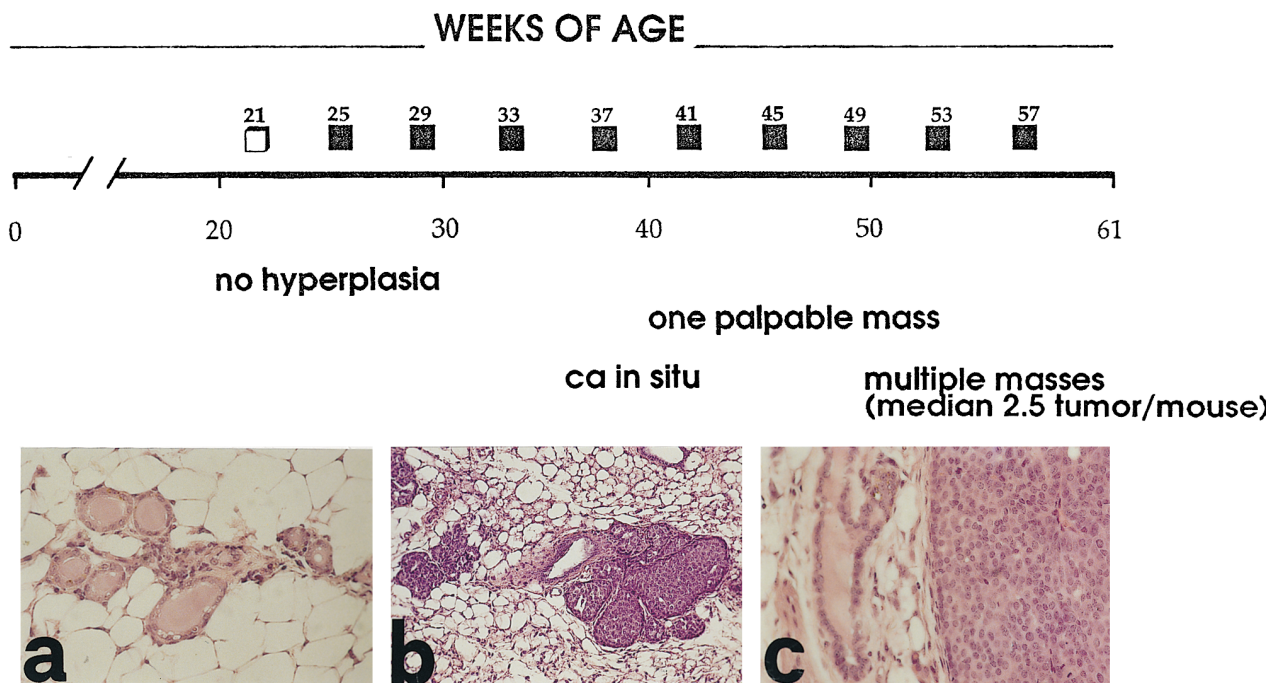
mRNA for Cytokine. Total RNA was prepared from carcinoma masses of mice treated or not with rIL-12 by using Ultraspec (Biotecx Lab. Inc., Houston, TX). 2 μg of RNA were reverse transcribed with Moloney murine leukemia virus reverse transcriptase (200 U) in 50 μl of reaction mixture with oligo dT and dNTP (GIBCO BRL, Paisley, UK). The cDNA were tested for the presence of murine glucose 3-phosphate dehydrogenase, IL-1α, IL-6, IFN-γ, TNF-α, GM-CSF, monokine induced by γ-IFN (MIG), and IFN-γ–inducible protein (IP-10) sequences in PCR reactions (Gene Amp Kit; Perkin Elmer Cetus, Norwalk, CT) performed in 50 μl volumes, by using specific primer pairs prepared by us (IP-10 and MIG) or from Clontech (Palo Alto, CA). The results are arbitrarily scored from $-$ to $+++$ based on the intensity of UV fluorescence of the ethidium bromide–stained gels as independently evaluated by two operators in a blind fashion.

Figure 1. Progression of HER-2/*neu* carcinogenesis in untreated BALB–NeuT and FVB–NeuN female mice. (*A*) Successive alterations of the mammary gland in untreated BALB–NeuT mice and indications of the time of 5-d courses of rIL-12 administration (□, 50 ng/day; ■, 100 ng/day). Histology shows that ductular atypical hyperplasia (*a*) is already evident and widely distributed in all mammary glands at 3 wk of age. This hyperplasia progresses to carcinoma in situ (*b*) between wk 13 and 17, and then to an invading lobular carcinoma (*c*). (*B*) Successive alterations of the mammary gland in FVB–NeuN mice during the 61-wk follow-up. Squares (□, ■, as in *A*). Histology shows that the mammary glands are normal (*a*) until at least wk 29 of age. Thereafter the neoplastic process begins in one mammary gland with focal hyperplasia and carcinoma in situ (*b*) and progresses by giving rise to an invasive lobular carcinoma (*c*) between wk 39 and 48. Original magnification: ×630.

A

BALB-NeuT female mice

_____ WEEKS OF AGE _____

| 2 | 5 | 9 | 13 | 17 | 21 | 25 | 29 |

0   5   10   15   20   25   30   33

atypical
mammary
hyperplasia

ca in situ   one
                    palpable mass

ten palpable
masses

B

FVB-NeuN female mice

_____ WEEKS OF AGE _____

| 21 | 25 | 29 | 33 | 37 | 41 | 45 | 49 | 53 | 57 |

0   20   30   40   50   61

no hyperplasia

one palpable mass

ca in situ   multiple masses
                    (median 2.5 tumor/mouse)

Statistical Analysis. Differences in tumor incidence were evaluated by the Mantel-Haenszel log-rank test, those in tumor/mouse numbers by Wilcoxon's rank sum test, and those in the number of tumor infiltrating cells by Student's *t* test.

Results

At week 33, lobular carcinomas were palpable in all 10 mammary glands of BALB–NeuT females carrying the activated HER-2/*neu* oncogene (Fig. 1 *A*). FVB–NeuN females carrying the HER-2/*neu* protooncogene display one or more mammary glands with tumor at 49 wk, and the mean number affected at 61 wk is still very low (Fig. 1 *B*). This rapid onset and total gland involvement suggests that expression of activated HER-2/*neu* in BALB–NeuT mice requires few, if any, additional genetic events to transform the mammary epithelial cell (7), whereas the delayed onset and asynchronous progression observed in the other line indicates that overexpression of HER-2/*neu* protooncogene results in stochastic tumor development.

These kinetic patterns provide two distinct models of HER-2/*neu* mammary oncogenesis with which to test the effect of prolonged administration of low doses of rIL-12. Since histologic examination revealed that widespread atypical hyperplasia was already evident in the mammary glands of 3-wk-old BALB–NeuT mice, and HER-2/*neu* mammary oncogenesis quickly progressed to overt carcinoma, these mice first received a 5-d course of 50 ng of rIL-12 plus MSA, injected intraperitoneally at 2 wk of age. The dose was then increased to 100 ng, and 5-d courses followed by 3 wk off were run from wk 5 to 29 (Fig. 1 *A*). MSA control mice received similar courses of MSA only. Mice were inspected weekly. Those displaying progressively growing masses in all 10 mammary glands and those that reached 33 wk of age were killed and morphologically examined.

A significant delay in the onset of the first mammary tumor was evident in the treated mice as compared with the MSA controls. Moreover, at wk 33 the number of mammary glands with a palpable tumor was much lower (Fig. 2, *top*). Histologic examination of the mammary glands at progressive time points in a fashion blind to the treatment revealed a general delay in the progression of the HER-2/*neu* oncogenesis in the treated mice. Atypical hyperplasia was less vigorous and less widely distributed than in the MSA controls, and no carcinoma in situ was found before wk 15 (data not shown). This delay in tumor progression was associated with deficient neovascularization and a marked increase in infiltrating CD8^{+}, and to a lesser extent CD4^{+}, lymphocytes (Table 1). Lymphocyte infiltration was concomitant with enhanced vascular cell adhesion molecule (VCAM)-1 expression (Fig. 3), as well as expression of mRNA for proinflammatory cytokines and their production (Table 1). Extensive expression of iNOS on tumors from treated mice was shown immunohistochemically (Fig. 3). This suggests that IL-12-primed NO exerted a cytotoxic and antitumor activity directly or through its reactive products (11, 12). Moreover, strong expression of IP-10
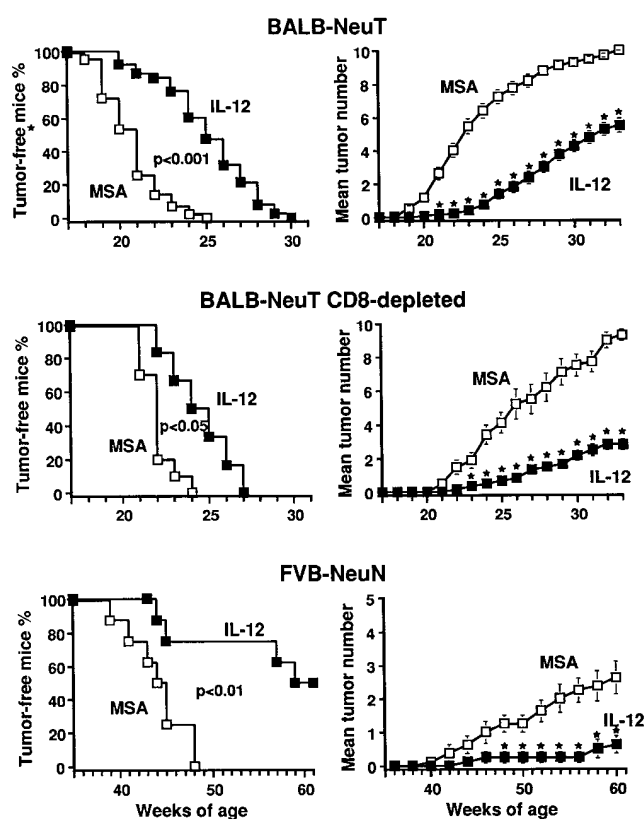

Figure 2. Inhibition of mammary carcinogenesis in HER-2/*neu* transgenic female mice treated with rIL-12. Percentage of tumor-free mice and mean number of palpable mammary carcinomas per mouse calculated as cumulative number of incident tumors/total number of BALB–NeuT mice, BALB–NeuT mice CD8^{+} T lymphocyte depleted by neonatal thymectomy and anti-CD8^{+} mAb treatment, and FVB–NeuN mice treated with rIL-12 (■) or MSA only (□). Stars (★) denote time points at which tumor multiplicity was significantly different (*P* <0.05 at least) between MSA and rIL-12 treatment groups. Group sizes were: BALB–NeuT: 38 MSA control mice and 43 rIL-12–treated mice; thymectomized and anti-CD8^{+} mAb treated BALB–NeuT mice: 10 MSA control mice, 6 rIL-12–treated mice; FVB–NeuN: 8 mice each group.

and MIG mRNA was revealed by reverse transcriptase PCR in the tumor area from treated mice (Table 1). These two chemokines mediate the antiangiogenic activity of IL-12, and, in combination, chemoattract activated T cells (13–15). Their modulation is probably a consequence of IL-12–induced expression of IFN-γ and TNF-α in NK and T cells. IFN-γ and TNF-α also amplify the production of proinflammatory cytokines, angiogenic inhibitors, and NO by reactive cells (15). This multifactorial scenario offers an explanation for both the inhibition of tumor neoangiogenesis and vascular damage leading to the impressive ischemic and hemorrhagic necrosis of advanced carcinomas in rIL-12–treated BALB–NeuT mice (Fig. 4).

Our previous studies have shown that CD8^{+} lymphocytes play a pivotal role in the antitumor reaction elicited by IL-12, whereas CD4^{+} cells play an inhibitory role (8, 16, 17). To evaluate the weight of CD8^{+} cells in rIL-12-delayed oncogenesis, BALB–NeuT mice treated with rIL-12 or MSA only were thymectomized at 4 wk and received

Table 1. *Effects of rIL-12 Treatment on Neovascularization, the Expression of Adhesion Molecules, the Secretion of Mediators, and the Infiltration by Reactive Cells in 7-mm Mean Diameter Tumors Growing in BALB–NeuT and FVB–NeuN Female Mice*

	BALB–NeuT mice		FVB–NeuN mice	
	MSA	MSA + IL-12	MSA	MSA + IL-12
Pathologic findings (immunohistochemistry)				
Microvessel count	15.9 ± 2.1	9.5 ± 1.3*	19.7 ± 3.7	10.5 ± 2.4*
Endothelial adhesion molecules				
ICAM-1	+	+	+	+
VCAM-1	−	++	−	++
ELAM-1	−	+/−	−	−
Infiltrating cells				
Macrophages	12.5 ± 4.1	13.4 ± 3.8	16.1 ± 4.2	14.2 ± 5.2
Granulocytes	5.2 ± 1.9	7.6 ± 2.3	5.0 ± 2.8	6.9 ± 3.0
CD8^{+} lymphocytes	2.9 ± 0.8	42.0 ± 8.3*	4.8 ± 2.1	14.6 ± 6.1*
CD4^{+} lymphocytes	3.1 ± 1.7	15.2 ± 5.1*	3.6 ± 1.9	5.7 ± 2.6
Cytokines and mediators				
IL-1β	−	+/−	−	+/−
TNF-α	+/−	+	+/−	+
IFN-γ	−	+	−	+
IL-6	+/−	+	+/−	+
iNOS	−	++	−	++
Reverse transcriptase PCR (mRNA)				
Cytokines and chemokines				
IL-1β	++	+++	NT[‡]	NT
TNF-α	−	+	NT	NT
IFN-γ	−	+/−	NT	NT
IL-6	++	++	NT	NT
GM-CSF	+/−	+	NT	NT
MIG	+	++	NT	NT
IP-10	+	++	NT	NT

Microvessel counts were performed on cryostat sections tested with an antiendothelial (CD31) mAb as described in Materials and Methods. The expression of adhesion molecules, cytokines, and mediators on cryostat sections was probed with the corresponding mAb. From mice with multiple tumors, two or more samples (one sample per tumor) were evaluated. At least 10 fields were counted per sample. Values are expressed as mean ± SD of five Balb–NeuT and four FVB–NeuN mice. An arbitrary score (from − to +++) was used to indicate the amount of detected signal in a semi-quantitative reverse transcriptase PCR analysis, as described in Materials and Methods. At least three independent tumors from Balb–NeuT mice were evaluated, and a representative expression pattern is shown.
*Value significantly different ($P < 0.001$) than that in MSA-treated mice.
[‡]NT, not tested.

200 μg of anti-CD8 mAb intraperitoneally. Both the progression of mammary oncogenesis and the protective effect of IL-12 were unaffected by this permanent CD8^{+} depletion (Fig. 2, *middle*).

The protective effect of rIL-12 was also evident in FVB–NeuN mice. Here, because of the slower tumor progression and the virtual absence of atypical mammary hyperplasia foci before wk 29, the initial 5-d course was administered to 21-wk-old mice (Fig. 1 *B*), followed the by same schedule as for the BALB–NeuT mice until wk 57. Mice displaying a tumor mass >2 cm mean diameter and those that reached the wk 61 of age were killed: 50% of the treated

mice were tumor-free, whereas all of the MSA controls displayed palpable tumors. The mean number of mammary glands with a palpable tumor was 2.6 per mouse in the MSA controls compared with only 0.9 in the IL-12–treated mice (Fig. 2, *bottom*). Deficient peri- and intratumoral vascularization, enhanced expression of adhesion molecules, enhanced CD8^{+} infiltration, cytokine production, and iNOS activation (Table 1 and Fig. 5), and large areas of hemorrhagic necrosis in established tumors (Fig. 4) were evident, as in the IL-12–treated BALB–NeuT mice.

Increased spleen and liver extramedullary hematopoiesis and a slight exacerbation of hepatic periportal mononuclear
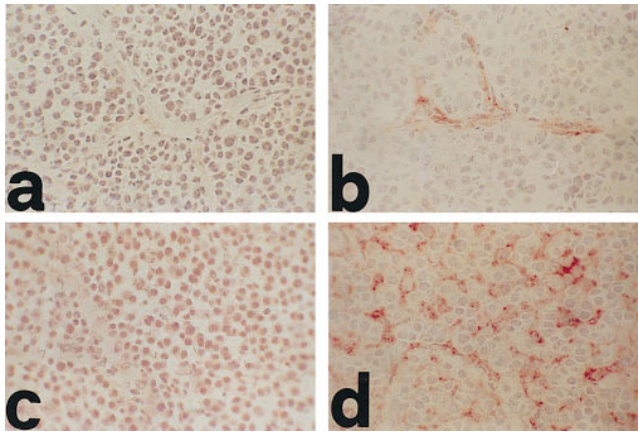
Figure 3. Cryostat sections of invading lobular carcinomas in MSA control (*a* and *c*) and IL-12–treated (*b* and *d*) BALB–NeuT mice. VCAM-1 is absent in tumor blood vessels of MSA control mice (*a*), whereas it is expressed in those of rIL-12–treated mice (*b*) as revealed by anti–VCAM-1 mAb. In the MSA control tumors, iNOS expression is almost undetectable in macrophages (*c*), whereas in rIL-12–treated mice it is clearly evident in macrophages intermingled among tumor cells (*d*) as shown by anti-iNOS mAb. Observations made in 7-mm mean diameter tumors. Similar patterns are evident in all established tumors. Original magnification: ×630.

Figure 5. Cryostat sections of invading lobular carcinomas in MSA control (*a* and *c*) and IL-12–treated (*b* and *d*) FVB–NeuN mice. TNF-α (*b*) and IFN-γ (*d*) are evident in the tumor growth area in IL-12–treated mice, whereas both are absent (*a* and *c*) in the MSA control, as revealed by staining with anti–TNF-α and –IFN-γ mAb in tumor masses of 49-wk-old mice. Original magnification: ×630.

infiltration were the only signs of chronic rIL-12–related lesions in both lines of mice, showing that this cytokine is effective at well-tolerated doses.

Discussion

rIL-12 administration in young BALB–NeuT mice carrying the activated Her-2/*neu* oncogene delays the appearance of tumor and reduces the number of mammary glands involved. In adult FVB–NeuN mice carrying the HER-2/
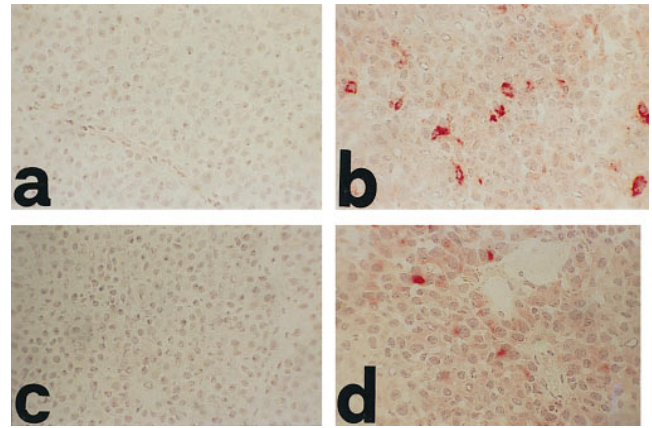
neu protooncogene, neoplastic progression is less impetuous and 50% of IL-12–treated mice are tumor-free at 1 yr. Since pathologic examinations suggest that rIL-12 does not lead to an appreciable failure in normal mammary tissue development, the increased percentage of FVB–NeuN–protected mice may mean that progression was delayed by rIL-12 beyond the 61 wk of the experiment.

The protection offered by rIL-12 could, of course, be ascribable to its triggered killing of neoplastic cells, thus offsetting the continuous generation of new transformed cells by the transgenic mammary tissue. However, in contrast with the rIL-12–induced immune reaction against established carcinomas (8), the results obtained with thymecto-
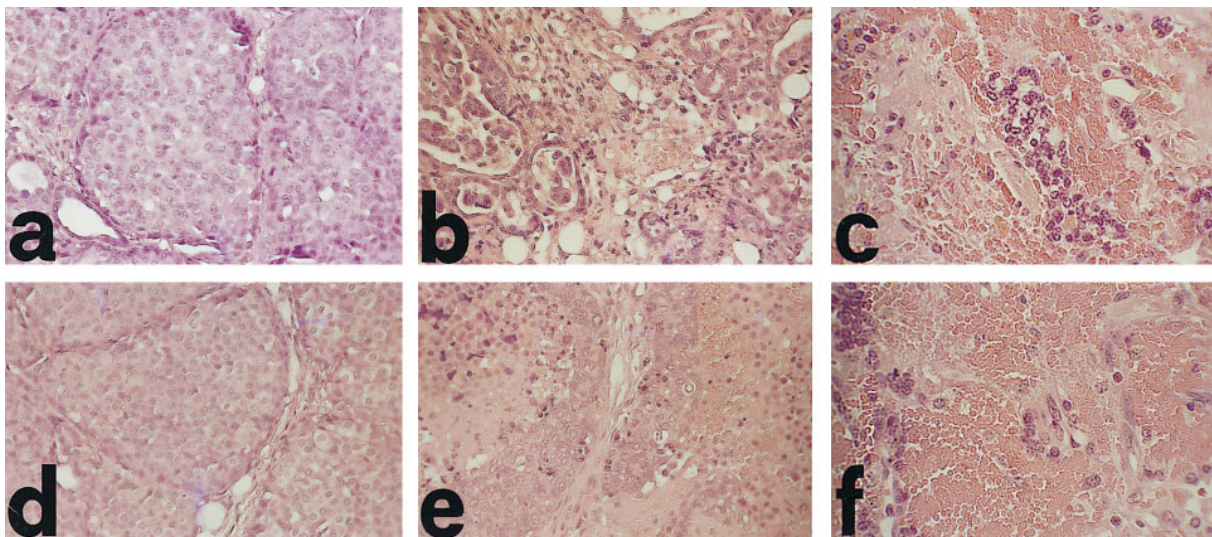

Figure 4. Histologic features of invading lobular carcinomas in BALB–NeuT (*a*) and FVB–NeuN (*d*) MSA control mice and in rIL-12–treated BALB–NeuT (*b* and *c*) and FVB–NeuN (*e* and *f*) mice. The impressive ischemic and hemorrhagic necrosis disaggregating the neoplastic masses becomes evident from wk 27 in BALB–NeuT (*b*) and wk 53 in FVB–NeuN (*e*) mice. In both cases it eventually results in extensive tumor destruction (*c* and *f*). Original magnification: ×630.

mized BALB–NeuT mice suggest that CD8$^+$ lymphocytes do not play a major role here, even if they are the dominant population of reactive cells infiltrating the tumor area in rIL-12–treated mice. It is possible that NK cells are the leading actors in this IL-12–provoked delay in oncogenesis (18). Since the NK1.1 mAb recognizes an allelic form of an NK cell antigen expressed by C57BL/6 but not BALB/c mice, the type and function of lymphocytes first involved are currently under investigation in (BALB/c × C57BL/6) NeuT$^+$ F1 mice.

The nonessential role of cytotoxic CD8$^+$ lymphocytes in vivo fits in well with both the marginal cytotoxic activity and the lymphokine release observed when T lymphocytes from rIL-12–treated mice were restimulated in vitro with APCs pulsed with the product of the rat HER-2/*neu* oncogene (p185), a self-protein in these transgenic mice (data not shown). These findings, along with morphologic data, rather suggest that rIL-12 essentially protects by halting angiogenesis (19) through the induction of several secondary cytokines and mediators, thus slowing down the transition from hyperplasia to overt carcinoma (20, 21). Moreover, the secondary cytokines and the tertiary chemokines and monokines elicited by IL-12 are apparently responsible for later vessel endothelial wall injury and hemorrhagic necrosis of established tumors (12, 13, 22–24). The endothelial vessel alteration and hemorrhagic necrosis observed in both lines of rIL-12–treated mice are reminiscent of those observed during rejection of an established transplantable mammary adenocarcinoma in BALB/c mice treated with systemic rIL-12 or adenocarcinoma cells engineered to release IL-12 (8).

Whether immunological maneuvers in healthy individuals at risk prevent the development of cancer is a question that has rarely been examined. Most studies have evaluated immunization before transplantation with frank tumor cells. Immunoprevention of cancer has been attempted in a few models of chemical carcinogenesis. Interpretation of these experiments may be confounded by the fact that carcinogens may be immunosuppressive.

Noguchi et al. have shown that similar doses of IL-12 inhibit 3-methylcholanthrene carcinogenesis in mice through the release of cytokines and mediators and nonspecific immune mechanisms (25). Our findings extend these observations by underscoring the potential of nonspecific immune mechanisms and their cross-talk with the endothelial cells of tumor vessels in the inhibition of HER-2/*neu* oncogenesis.

The HER-2/*neu* oncogene is expressed in a substantial proportion of human mammary carcinomas and its product seems to be a promising target for specific immune intervention (26). However, its expression in humans is not the same as the expression of a xenogenic oncogene in transgenic mice, and this issue may somewhat impact specific immunization rather than nonspecific immune mechanisms. The close resemblance of the progression of mammary carcinogenesis in HER-2/*neu* transgenic mice to that in women suggests that chronic administration of nontoxic rIL-12 regimens may be a significant prophylactic strategy. The direct proportionality between the length of carcinogenesis progression and the efficacy of rIL-12 suggest that a "soft" immunologic alternative to controversial and distasteful mastectomy may be envisaged.

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