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The Combined Action of IL-15 and IL-12 Gene Transfer Can Induce Tumor Cell Rejection Without T and NK Cell Involvement

Emma Di Carlo,* Alberto Comes, † Stefania Basso, † Alessandro De Ambrosis, † Raffaella Meazza,‡ Piero Musiani,* Karin Moelling,§ Adriana Albini, † and Silvano Ferrini 2†

The cooperative antitumor effects of IL-12 and IL-15 gene transfer were studied in the N592 MHC class I-negative small cell lung cancer cell line xenotransplanted in nude mice. N592 cells engineered to secrete IL-15 displayed a significantly reduced tumor growth kinetics, and a slightly reduced tumor take rate, while N592 engineered with IL-12 displayed only minor changes in their growth in nude mice. However, N592 cells producing both cytokines were completely rejected, and produced a potent local bystander effect, inducing rejection of coinjected wild-type tumor cells. N592/IL-12/IL-15 cells were completely and promptly rejected also in NK-depleted nude mice, while in granulocyte-depleted animals a slight delay in the rejection process was observed. Immunohistochemical analyses of the N592/IL-12/IL-15 tumor area in intact nude mice revealed the presence of infiltrating macrophages, granulocytes, and NK cells, and expression of inducible NO synthase and of secondary cytokines such as IL-1β, TNF-α, and IFN-γ, and at higher levels GM-CSF, macrophage-inflammatory protein-2, and monocyte chemoattractant protein-1. In NK cell-depleted nude mice, numerous macrophages and granulocytes infiltrated the tumor, and a strong expression of macrophage-inflammatory protein-2 and inducible NO synthase was also observed. Finally, macrophages cocultured with N592/IL-12/IL-15 produced NO in vitro, and inhibited tumor cell growth, further suggesting their role as effector cells in this model. The Journal of Immunology, 2000, 165: 3111–3118.

The importance of MHC class I-restricted CTLs as effectors of antitumor immunity has been widely demonstrated, and several CTL-defined tumor-associated Ags, representing potential targets for a tumor-specific immunotherapy, have been identified (1–3). However, a major tumor escape mechanism that hampers the development of CTL-based immunotherapy is the lack or the loss of MHC class I expression in a significant fraction of human tumors (4–6). The loss of individual alleles can be the consequence of an in vivo selective pressure by class I-restricted CTLs (7). MHC class I defects may depend on genomic deletion or mutation of MHC or MHC-related genes in some tumor cell types (4, 6). In other tumors, a down-regulation of MHC class I molecules has been related to down-regulation of MHC class I heavy chains (8), β2-microglobulin (9), or TAP expression (10, 11). In tumors lacking MHC class I, antitumor functions may be exerted by effectors of natural immunity that can be regarded as potential tools for antitumor immunotherapy strategies (12).

Among natural immunity effectors, NK cells are known to recognize and lyse cells that lack expression of MHC class I (13, 14). In fact, the expression of MHC class I on normal cells confers protection from NK cell lysis, through NK-inhibitory receptors for MHC class I (15, 16). In fact, the expression of MHC class I on normal cells confers protection from NK cell lysis, through NK-inhibitory receptors for MHC class I (15, 16). Thus, down-regulation of MHC class I expression in tumor cells results in enhanced NK susceptibility.

IL-15 is a four α-helix bundle cytokine displaying IL-2-like immunostimulatory functions (17, 18), supporting the proliferation and differentiation of T, B, and NK cells. In addition, IL-15 has been reported to control differentiation of NK cells from bone marrow precursors (19), to stimulate NK antitumor cytolytic functions (20, 21), and to act as a chemotactic stimulus for NK cells (22). The critical role of IL-15 in NK cell development and function was also evidenced in IFN-regulatory factor 1 (IRF-1) knockout mice, which lack IL-15 expression and display an NK-deficient phenotype (23). In view of these properties, IL-15 has been regarded as a suitable candidate for cancer immunotherapy (24) or gene therapy strategies (25, 26).

In a previous study, we have shown that a human MHC class I-negative tumor, engineered to secrete IL-15, displayed a reduced growth and take rate when xenotransplanted in nude mice, although tumorigenicity was not completely abrogated (27). High number of infiltrating NK cells were found at the tumor site, a finding that has been rarely observed in cytokine-transduced tumors (28), and the IL-15-mediated effects were abrogated by NK cell depletion. We speculated that the combined gene transfer of IL-15 with other NK-stimulating factor(s) could synergistically enhance the antitumor effects of NK cells recruited by IL-15 at the tumor site.

IL-12 is a heterodimeric cytokine, secreted by monocytes, macrophages, and dendritic cells, which is able to activate both T and NK cell functions. Thus, IL-12 is a potent inducer of Th1 responses and induces NK cell proliferation, cytotoxic activity, and IFN-γ production (29). Several studies have demonstrated a potent
antitumor activity of IL-12 either as a recombinant cytokine or in gene transfer approaches in different syngeneic mice models (29–31). Another report showed that the combination of IL-12 and IL-15 synergistically potentiated cytokine production by NK cells in vitro (32). In addition, a combination of suboptimal doses of the two recombinant cytokines induced antitumor effects in a B16 melanoma model in syngeneic mice (33).

In this study, we have analyzed the possible cooperative antitumor effects of IL-12 and IL-15 gene transfer in a human small cell lung cancer cell line, N592, chosen as a prototype of MHC class I-negative tumor. The effects on natural immunity were studied by xenotransplantation in nude mice. Interestingly, engineering of tumor cells with both IL-15 and IL-12 resulted in a complete tumor rejection also in NK-depleted or in granulocyte-depleted nude mice, suggesting a predominant role of macrophages in this model.

Materials and Methods

Cell lines and cultures

N592 small cell lung cancer cell line was kindly provided by Dr. J. Minna, National Cancer Institute (Washington, D.C.). Cells were cultured in endotoxin-free RPMI 1640 medium (endotoxin content <0.005 EU/ml) supplemented with l-glutamine and antibiotics (all from BioWhittaker, Bergamo, Italy) and 10% FCS (endotoxin content 20 EU/ml; Seromed Biochrom, Berlin, Germany).

Plasmid vector assembly and N592 transfection

The pVL/KL-15RESneo plasmid vector was obtained as previously described (27, 34). The bicistronic mIL-12 cDNA, encoding for p40 and p35 murine IL-12 chains, was digested from pRES-muIL-12 (35) with BamH1 and was subcloned in pIREsneo or pIREsHygro plasmid vectors (Clontech Laboratories, Palo Alto, CA). The orientation of the insert was checked by further digestion with BstXI restriction enzyme. N592 cells were transfected with 10 μg of pVL/KL-15RESneo or pmuIL-12RESHygro plasmids or both using cationic liposomes (DOTAP; Boehringer/Roche, Milano, Italy). Stable transfectants and clones were obtained by limiting dilution, in medium containing either G418 (500 μg/ml) or hygromycin (250 μg/ml) or both, and were then tested for IL-15 and IL-12 production.

IL-15, IL-12, GM-CSF, and IFN-γ ELISA or bioassays

As indicator cell system for determination of IL-2/IL-15 activity, we used the CTLL mouse cell line, known to proliferate in response to human IL-2 or IL-15. Cytokine activity was assessed by [3H]thymidine uptake by CTLL after 6-h pulse with 0.5 IU of human rIL-2 or human rIL-15 (Genzyme, Cambridge, MA) containing a known antitumor activity of IL-15 and IL-12. Cytokine activity was assessed by [3H]thymidine uptake by CTLL after 6-h pulse with 0.5 IU of human rIL-2 or human rIL-15 (Genzyme, Cambridge, MA) containing a known

Isolation of granulocyte and macrophage populations, production of NO, and cytotoxicity

To isolate granulocytes, nude mice were injected i.p. with 1 ml of 9% sodium casein (Sigma-Aldrich, Milano, Italy) in endotoxin-free PBS, followed by a second injection 16 h later. Three hours later, peritoneal cells were recovered in 5 ml of DMEM containing 0.5 mM EDTA. To isolate macrophages, nude mice were injected i.p. with 1 ml of 2.9% aged thioglycolate (Difco, Detroit, MI) solution. After 2 days, peritoneal cells were harvested as above. Cell populations were fractioned by self-forming 90% Percoll (Pharmacia Biotech, Uppsala, Sweden) gradients by centrifugation at 60,000 g for 20 min at 4°C, which produced a two-layer fractionation pattern. Macrophages were harvested from the upper layer of thioglycolate-induced peritoneal cell populations, while granulocytes were collected from the lower layer of casein-induced cells. Purity of each cell population was >90%, as judged by microscopic examination of stained cytospin preparations.

Macrophages or granulocytes were cocultured with different N592 transfectants (2 × 10⁶ in 1 ml of medium) at 10:1 or 20:1 ratios in 24-well plates. NO production in the supernatant was measured after 2 or 5 days by a colorimetric assay using the Griess reagent (Sigma-Aldrich) (36). Cytotoxicity against N592 transfectants, which grow in suspension, was evaluated by gently resuspending N592 and transferring them in triplicate wells of a 96-well flat-bottom plate. The amount of viable cells was evaluated by a standard MTT (Sigma-Aldrich) assay (37).

*Abbreviations used in this paper:* MCP, monocyte chemoattractant protein; iNOS, inducible NO synthase; L-NAME, Nω-nitro- L-arginine methyl ester; MIP, macrophage-inflammatory protein.
Results
Characterization of N592 transfectants secreting IL-12, IL-15, or both cytokines

N592 cells were transfected with pVkL/IL-15-IRESneo and/or pIL-12-IREShygro, either alone or in combination, and cloned after appropriate drug selection. The modified IL-15 cDNA VkL/IL-15 encodes for an IL-15 preprotein bearing the Ig L chain signal peptide that allows enhanced secretion of biologically active IL-15, upon transfection, as compared with unmodified IL-15 cDNAs (34). The N592 clones listed in Table I were selected for further studies because they displayed similar growth kinetics in vitro and on the basis of their cytokine secretion pattern. Thus, the N592/IL-12/IL-15 clone secreted amounts of IL-15 or IL-12 similar to that produced by the clones expressing only IL-15 or IL-12. IL-15 secreted by transfectants was biologically active in sustaining both IL-2/IL-15-sensitive CTL-L proliferation and in boosting NK cytolytic activity (27).

Coexpression of IL-12 and IL-15 synergistically induces antitumor effects in nude mice

The effect of cytokine engineering on tumorigenicity was evaluated by heterotopic (s.c.) implant in nude mice possessing a functional natural immunity. As shown in Fig. 1A, mock-transfected N592 (N592/neo/hygro) showed a very rapid growth kinetics in 100% of injected animals, which was similar to that of unmodified N592. N592/IL-12 cells displayed only minor changes of the growth pattern, while N592/IL-15 showed a clearly reduced tumor growth rate and a slight reduction in tumorigenicity (80% tumor take). However, N592/IL-12/IL-15 produced only a transient tumor growth, followed by complete rejection in all animals tested. Consistent results were obtained in three unrelated experiments.

Next we investigated possible inhibitory effects of N592/IL-12/IL-15 on the growth of wild-type tumor cells. Coinjection of N592/IL-12/IL-15 with a tumorigenic dose of N592pc at the same site induced only a transient tumor growth, followed by complete rejection in 100% of the animals, while injection of N592pc contralaterally to N592/IL-12/IL-15 produced tumor growth with a slightly reduced kinetics (Fig. 1B). Thus, a very potent local bystander effect on N592pc was produced by s.c. injection of N592/IL-12/IL-15. The weak systemic bystander effect, observed in animals injected with N592pc contralaterally to N592/IL-12/IL-15 may be related to the low levels of circulating IL-15 (15 pg/ml at day +3, undetectable at day +7). In contrast, consistent levels of IL-12 (1600 pg/ml) were found at day +3, and it was still detectable at day +7.

Effect of NK cell and granulocyte depletion on N592 transfectant tumor growth

To gain further information on the role of NK cells, N592/IL-15 and N592/IL-12/IL-15 transfectants were injected in nude mice that had been treated with anti-asialo GM1 antiserum. N592/IL-15 and N592/IL-12 showed growth kinetics similar to that of N592pc in NK-depleted nude mice, while N592/IL-12/IL-15 were still completely rejected (Fig. 2). Treatment with nonimmune rabbit control Ig had virtually no effect (Fig. 2B). These findings indicate that NK cells are mainly responsible for the antitumor effect of IL-15, while these cells are not necessary for the IL-15/IL-12 cooperative effect. In addition, N592/IL-12/IL-15 were also rejected in granulocyte-depleted nude mice, although a delay in the rejection process was observed in these animals (Fig. 2C). Similar results were observed in nude mice depleted of both NK cells and

<table>
<thead>
<tr>
<th>Cells</th>
<th>IL-12 (pg/ml)</th>
<th>IL-15 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N592 pc</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N592/IL-12</td>
<td>14,560 ± 2,036</td>
<td>0</td>
</tr>
<tr>
<td>N592/IL-15</td>
<td>0</td>
<td>359 ± 40</td>
</tr>
<tr>
<td>N592/IL-15/IL-12</td>
<td>14,040 ± 1,095</td>
<td>405 ± 35</td>
</tr>
</tbody>
</table>

* Data refer to the mean concentration of cytokine in the 48-h supernatants of three subconfluent cultures (5 × 10⁶ cell/ml) of each transfectant, as detected by ELISA.
granulocytes (data not shown), thus suggesting that macrophages play a predominant role.

**IL-12 and IL-15 synergistically stimulate IFN-γ and GM-CSF secretion by murine splenocytes in vitro**

We further analyzed whether IL-15 and IL-12 produced by transfectants could synergize in inducing cytokine production by mouse splenocytes in a coculture system. As shown in Fig. 3A, after 3 days of coculture with N592/IL-12/IL-15, splenocytes released 1500 pg/ml of IFN-γ in the supernatant, vs average levels of 10 and 110 pg/ml measured in N592/IL-15 and N592/IL-12 coculture supernatants, respectively. Similar results were obtained for GM-CSF (Fig. 3B). Thus, 15 and 50 pg/ml of GM-CSF were found in cocultures with N592/IL-15 and N592/IL-12, respectively, while 100 pg/ml was secreted in N592/IL-12/IL-15 cocultures. This cooperative effect of IL-15 and IL-12 was largely dependent on NK cell stimulation because splenocytes from anti-asialo GM1-depleted animals produced lower levels of IFN-γ or GM-CSF (Fig. 3, A and B), as compared with splenocytes from untreated animals or from animals treated with control rabbit Ig (not shown).

**Histological and immunohistochemical analysis of the rejection process**

To gain further information on the mechanisms underlying the cooperative effects of transfected IL-15 and IL-12 in vivo, we performed histological and immunohistochemical analysis of the N592pc, N592/IL-12, N592/IL-15, and N592/IL-12/IL-15 tumor area in intact or NK-depleted nude mice.

When injected in nude mice, either N592/pc or N592/neo/hygro cells gave rise to a richly vascularized tumor arranged in small alveolar structures with few infiltrating macrophages (Fig. 4a). A similar growth pattern was observed in N592/IL-12 tumor growth area in which, however, microvessels were less numerous and a few necrotic areas were present (Fig. 4d). The periphery of
cytokines were almost absent (Table II). Vascularization was scarce to absent with diffusely damaged microvessel sprouts. Production of proinflammatory cytokines was quite similar to that observed in N592/IL-12 tumor growth area, while production of GM-CSF, MIP-2, and MCP-1 was stronger.

In NK cell-depleted nude mice, N592/IL-12/IL-15 tumor rejection area was markedly infiltrated by macrophages and granulocytes with a moderate production of IL-1β, TNF-α, and MCP-1, and a strong and widely distributed expression of iNOS and MIP-2 (Fig. 5, a–e, and Table II). Anti-endothelial cell (anti-CD31) staining was scarce to absent with a dusty appearance, indicating the heavily injured endothelial cells (Fig. 5f).

**NO production and cytotoxicity of granulocyte- or macrophage-enriched populations**

In the attempt to identify the cells responsible for NO production, we first isolated thioglycolate-induced macrophages or casein-elicited granulocyte fractions by density gradients and tested their ability to produce NO in coculture with N592/IL-12/IL-15. As shown in Fig. 6A, only macrophage-enriched populations released significant amounts of NO in response to coculture with N592/IL-12/IL-15, while granulocyte-enriched fractions did not (Fig. 6A). When macrophages and granulocytes were cocultured together with N592/IL-12/IL-15, a slight cooperative effect in NO production was observed.

Although N592/IL-12 also induced some NO production by macrophages, induction by N592/IL-12/IL-15 had a clearly stronger effect (Fig. 6B). In addition, macrophage-enriched populations markedly inhibited N592/IL-12/IL-15 growth in a 5-day MTT assay, while N592pc were not affected, and N592/IL-12 and N592/IL-15 were only partially inhibited (Fig. 6C). The finding that N592/IL-15 growth was partially inhibited by macrophages, in the absence of NO production, suggested that both NO-dependent and NO-independent mechanisms of tumor cell growth inhibition were operative. This possibility was also suggested by the use of the iNOS inhibitor l-NAME, (N-nitro-L-arginine methyl ester) which potently inhibited NO production by macrophages at 5 mM, but only partially blocked cytotoxicity (Fig. 6D) against N592/IL-12/IL-15.

**Discussion**

In this study, we show that the combination of IL-12 and IL-15 gene transfer in a human MHC class I-negative tumor synergistically triggers tumor rejection by natural immunity in nude mice. Although NK cells accumulate at the site of rejection of tumor cells engineered to secrete both cytokines, tumor rejection occurs also in animals that had been depleted of NK cells and of granulocytes, through the involvement of macrophages.

Although IL-12 has been proven to exert antitumor effects in a number of experimentally induced and spontaneous tumors in immunocompetent hosts (29–31), it had virtually no efficacy in our tumor gene transfer model in nude mice. In the absence of T lymphocytes, N592/IL-12 tumor growth kinetic was not affected despite an evident aspecific reactive cell infiltration and proinflammatory cytokine production at the tumor site. Furthermore, small sprouting vessels, mainly found at the periphery of tumor mass, may support tumoral cell survival, making easier tumor escape from any attempt of host natural immune response. Altogether, these observations suggest the requirement of T cells in leading to an efficient IL-12-induced antitumor reaction. Furthermore, a pivotal role of CD8* cells has been found in the rejection of IL-12.

**FIGURE 4.** Histologic (a, d, g, j) and immunohistochemical (b, c, e, f, h, i, k, l) features of 7-day N592pc, N592/IL-12, and N592/IL-15 tumors, and 5-day N592/IL-12/IL-15 tumor in nude mice. N592pc cells gave rise to a well-vascularized tumor arranged in small alveolar structures (a) with no infiltrating NK cells (b) and few granulocytes (c). N592/IL-12 tumor showed a few necrotic areas (d) and a moderate peripheral infiltrate of NK cells (e) and PMNs (f). N592/IL-15 tumor showed large necrotic areas (g) with numerous NK cells (h) and several granulocytes (i). N592/IL-12/IL-15 tumor rejection area consisted in a large area of ischemic necrosis bordered by the few and injured tumor cell aggregates (j) richly infiltrated by granulocytes (k) and NK cells (l).
corresponding Abs. IFN-γ rect angiogenesis inhibition by secondary cytokines (mainly correlated with the level of IFN-γ IL-12 antitumor effect, induced by subtherapeutic doses of recombinant IFN-γ IL-15-induced NK cell production of several cytokines such as GM-CSF and IL-15 on NK and lymphokine-activated killer cytotoxins (40), with the reciprocal up-regulation of receptor expression. Our data revealed a marked macrophage and granulocyte recruitment, as well as the presence of iNOS and NO production. In agreement with these previous observations, immune cells strongly produced MIP-2, under the influence of cytokines present in C57BL/6 mice. Furthermore, expression of cytokines was defined as absent (−), scarcely (+), moderately (++), frequently (+++), or strongly (++++) present on cryostat sections tested with the corresponding Abs.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>N592pc</th>
<th>N592/IL-12</th>
<th>N592/IL-15</th>
<th>N592/IL-12/IL-15</th>
<th>N592/IL-12/IL-15*</th>
</tr>
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<tbody>
<tr>
<td>IL-1β</td>
<td>–</td>
<td>±</td>
<td>±</td>
<td>±</td>
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<tr>
<td>TNFa</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
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<tr>
<td>IFNγ</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>GM-CSF</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>–</td>
</tr>
<tr>
<td>MIP-2</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>MCP-1</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>++</td>
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<tr>
<td>iNOS</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>++</td>
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</tbody>
</table>

* Values significantly different (p < 0.001) from corresponding values in N592pc.

Although two isoforms of IL-15 mRNA are constitutively expressed in human tumors (34, 38), spontaneous secretion of IL-15 by tumor cells was not observed due to the existence of multiple posttranscriptional levels of IL-15 production control (18, 39). We previously reported that the use of a modified human IL-15 cDNA for gene transfer allowed secretion of IL-15 that was found to be biologically active on both human and murine NK and T cells (34). Injection of IL-15-engineered N592 tumor cells in nude mice resulted in an NK cell-dependent inhibition of tumor growth. In the light of the synergistic or additive effects exerted by low doses of IL-12 and IL-15 on NK and lymphokine-activated killer cytotoxities (40), with the reciprocal up-regulation of receptor expression on mononuclear cells (33, 41), one may expect an NK cell-mediated rejection of N592 cells releasing both cytokines. In addition, it has been reported that IL-15 synergistically potentiates IL-12-induced NK cell production of several cytokines such as IFN-γ, IL-10, MIP-1α, and MIP-1β (32). Moreover, a cooperative antitumor effect, induced by subtherapeutic doses of recombinant IL-12 + IL-15 in a B16F10 melanoma model in syngeneic mice, correlated with the level of IFN-γ production (33). In agreement with these previous observations, immune cells strongly produced MIP-2 and NO production. Infiltrating reactive cells strongly produced MIP-2 and NO (e). Immunostaining with anti-endothelial cell (CD31) Ab (f) revealed a dusty appearance of positive cells, indicating the scattered heavily injured endothelial cells.

**FIGURE 5.** Histologic (a) and immunohistochemical (b–f) features of N592/IL-12/IL-15 tumor area rejection, 5 days after s.c. tumor cell injection in NK cell-depleted nude mice. An extensive necrosis of colliquative type characterized the area of tumor cell rejection (a) in which numerous macrophages (b) and granulocytes (c) were present. Infiltrating reactive cells strongly produced MIP-2 (d) and NO (e). Immunostaining with anti-endothelial cell (CD31) Ab (f) revealed a dusty appearance of positive cells, indicating the scattered heavily injured endothelial cells.
In contrast, granulocytes, attracted at the tumor site by MIP-2 (murine equivalent of IL-8) (42), may also cooperate in NO production (42–45), and in tumor destruction (46–50). Both granulocytes and macrophages could also be responsible for the blood vessel injuries observed in N592/IL-12/IL-15 tumor (29, 50). In addition, it should be outlined that human neutrophils can respond to IL-12 stimulation (51) and can also be activated by IL-15 to express IL-8 (52). Indeed, granulocytes appeared to play a cooperative role in our model because in granulocyte-depleted animals, the N592/IL-12/IL-15 rejection process was delayed.

In NK cell-depleted nude mice, it is likely that the cooperative biological effects of IL-12 and IL-15 mainly target macrophages and granulocytes, allowing the onset of a distinct natural immunity regulatory network. In this context, an increased iNOS and MIP-2 expression, detected at the tumor site, should be viewed as signs of an increased IL-12 and/or IL-15 direct macrophage and granulocyte activation (51–54). Although NK-depleted nude mice showed a reduced IFN-γ production in response to IL-12 + IL-15 produced by transfectants, both in vitro and in vivo, it is likely that low amounts of macrophage-derived IFN-γ (53) may play a role, particularly in iNOS induction. Thus, low amounts of IFN-γ were found in cocultures of peritoneal macrophages with double-transfected cells (data not shown).

Different from previous studies in tumor cytokine immuno/gene therapy, which emphasize the requirement of a cross-talk between specific and nonspecific immune mechanisms to obtain a complete tumor rejection (55, 56), in this study we report evidence that the activation of macrophages by the synergistic action of IL-12 + IL-15 may lead to an effective T- and NK-independent tumor growth inhibition. These data provide new insight on the cooperative activity of IL-12 and IL-15 in the stimulation of effective natural immunity response and could be relevant for the development of gene therapy strategies against MHC class I-negative tumors escaping from specific CTL control.

Acknowledgments
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T cells on two successive metastases of a melanoma patient are consistent with immune selection: activation with IL-12 followed by IL-15 stimulation. ImmunoL.13:851.


