Induction of Antitumor Immunity by Vaccination of Dendritic Cells Transfected with MUC1 RNA

Shigeo Koido, Masahiro Kashiwaba, Dongshu Chen, Sandra Gendler, Donald Kufe and Jianlin Gong

*J Immunol* 2000; 165:5713-5719; doi: 10.4049/jimmunol.165.10.5713

http://www.jimmunol.org/content/165/10/5713

**References**
This article cites 57 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/165/10/5713.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Induction of Antitumor Immunity by Vaccination of Dendritic Cells Transfected with MUC1 RNA

Shigeo Koido,* Masahiro Kashiwaba,* Dongshu Chen,* Sandra Gendler,† Donald Kufe,* and Jianlin Gong2*†

Dendritic cells (DC) are potent APCs. In this study, murine bone marrow-derived DC were transfected with RNA encoding the MUC1 Ag that is aberrantly overexpressed in human breast and other carcinomas. The MUC1 RNA-transfected DC exhibited cell surface expression of MUC1 and costimulatory molecules. After injection at the base of the tail, the transfected DC were detectable in inguinal lymph nodes by dual immunohistochemical staining. Vaccination of wild-type mice with MUC1 RNA-transfected DC induced anti-MUC1 immune responses against MUC1-positive MC38/MUC1, but not MUC1-negative, tumor cells. Mice immunized with the transfected DC were protected against challenge with MC38/MUC1 tumor cells. Furthermore, mice with established MC38/MUC1 tumors were eliminated after receiving the vaccination. CTLs isolated from mice immunized with the transfected DC exhibited specific cytolytic activity against MC38/MUC1 tumor cells. In contrast to these findings, there was little if any anti-MUC1 immunity induced with the transfected DC in MUC1 transgenic (MUC1.Tg) mice. However, coadministration of the transfected DC and IL-12 reversed the unresponsiveness to MUC1 Ag in MUC1.Tg mice and induced MUC1-specific immune responses. These findings demonstrate that vaccination of DC transfected with MUC1 RNA and IL-12 reverses tolerance to MUC1 and induces immunity against MUC1-positive tumors. The Journal of Immunology, 2000, 165: 5713–5719.

Dendritic cells (DC) are potent APCs essential for the initiation of primary immune responses (1, 2). DC derive their potency by abundant expression of MHC class I, class II, costimulatory, and adhesion molecules, which provide secondary signals for the stimulation of naive T cell populations (3–5). DC-based vaccines can directly activate a CTL response and thus elicit a more potent antitumor immune response than genetically modified tumor cell-based vaccines. Various strategies have been reported to introduce tumor Ags into DC to generate CTL responses against murine and human malignant cells (6–11). DC loaded with tumor Ags, peptides, DNA, or lysates have induced CTLs and antitumor immunity (6, 7, 10, 12).

DC transfected with RNA derived from tumor cells have also been used as vaccines (13, 14). DC transfected with chicken OVA RNA stimulate primary anti-OVA CTL responses in vitro. Moreover, vaccination of mice with OVA RNA-transfected DC has been used to treat OVA-positive tumor metastases to lung (13). Other studies using human DC transfected with carcinoembryogenic Ag RNA have demonstrated effective generation of Ag-specific CTL in vitro (14, 15). An advantage of using RNA from tumors, rather than peptides, is that RNA encodes multiple epitopes for presentation by diverse HLA alleles. In addition, DC that are transfected with RNA encoding a tumor Ag or RNA extracted from patient-derived tumor endogenously express the tumor Ag by translation of the transfected RNA. The tumor Ag is then subject to endogenous processing by the DC and presentation of peptides to induce a T cell response (16–18).

The human DF3/MUC1 Ag is aberrantly overexpressed in breast, ovarian, pancreatic, and other carcinomas (19, 20). The heavily glycosylated MUC1 protein is normally expressed by epithelial cells at apical borders lining the lumen of duct (21, 22). By contrast, in transformed epithelial cells, MUC1 is expressed at high levels over the entire cell surface. Moreover, defective glycosylation of the MUC1 protein in transformed epithelial cells has been associated with unmasking of cryptic epitopes. Immunity against MUC1 has been identified in certain patients with tumors (23). Non-MHC-restricted cytotoxic T cell responses to MUC1 have been reported in patients with breast, pancreatic, and ovarian cancers (24–28). In addition, Ab responses to MUC1 have been found in patients with ovarian cancer (29). These findings suggest that MUC1 represents a potential target for active specific immunotherapy of certain human tumors.

The present studies have assessed the effects of transfecting MUC1 RNA into DC as a vaccine for the induction of immunity against MUC1-positive tumors. The results demonstrate that DC transfected with MUC1 RNA (DC/MUC1 RNA) induce protection against challenge of wild-type mice with MUC1-positive MC38 carcinoma cells. We also demonstrate that vaccination with DC/MUC1 RNA and coadministration of IL-12 reverses immunologic unresponsiveness to MUC1 Ag in MUC1-transgenic (MUC1.Tg) mice.

Materials and Methods

Mice

Female wild-type C57BL/6 mice were purchased from Taconic Farms (Germantown, NY). C57BL/6 MUC1-transgenic mice (MUC1.Tg) were housed and maintained in microisolator cages under specific pathogen-free...
Synthesis of mRNA transcripts

An XhoI fragment containing a full-length MUC1 cDNA with seven tandem repeats was cloned into the XhoI site of the pcDNA3 plasmid under control of the T7 RNA polymerase promoter (pcDNA3/MUC1) (32, 33). Clones containing the MUC1 cDNA were isolated, and large scale preparations were generated using Maxi Prep kits (Qiagen, Valencia, CA). The plasmid was then linearized and transcribed using the mCap TM RNA Capping kit (Stratagene, La Jolla, CA). The linearized DNA template was removed by digestion with DNase, and mRNA transcripts were purified by phenol/chloroform extraction followed by ethanol precipitation. Capped mRNA was polyadenylated by yeast poly(A) polymerase (Amersham, Piscataway, NJ). Capped, polyadenylated MUC1 mRNA was recovered by phenol/chloroform extraction and ethanol precipitation. As a control, capped, polyadenylated β-galactosidase mRNA was also synthesized in vitro.

Cell culture and DC generation

Murine (C57BL/6) MC38 adenocarcinoma cells were stably transfected with MUC1 cDNA (MC38/MUC1) (34, 35). The MC38, MC38/MUC1 tumor cells, and MCF7 human breast carcinoma cells (MUC1 positive; American Type Culture Collection, Manassas, VA) were maintained in DMEM supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. DC were generated from bone marrow of wild-type C57BL/6 mice as previously described (36). Briefly, bone marrow was flushed from the long bones and depleted of lymphocytes, granulocytes, and Ia– cells using mAbs 2.4.3 (anti-CD8; American Type Culture Collection), GK1.5 (anti-CD4; American Type Culture Collection), RA3-1A/6.1 (anti-B220; CDS4SR; American Type Culture Collection), B2L.1 (anti-IA; American Type Culture Collection) RB6-8C5 (anti-Gr-1; Pharmingen, San Diego, CA), and rabbit complement. Cells were resuspended in RPMI 1640 medium containing 15 ng/ml recombinant murine GM-CSF (Sigma, St. Louis, MO). On day 6 of culture, nonadherent and loosely adherent cells were collected and replated in 100-mm culture dishes (106 cells/ml; 10 ml/dish). The nonadherent cells were removed after 30 min by washing, and medium containing GM-CSF was added to the dish. The cells were incubated for 24 h, and nonadherent cells (DC) were removed for transfection with RNA.

Transfection of DC

DC were collected on day 7 and washed twice in serum-free Opti-MEM medium (Life Technologies, Grand Island, NY). DC were resuspended in Opti-MEM at 1 x 10^7 cells/ml in 50-ml Falcon tubes (Becton Dickinson, Franklin Lakes, NJ). MUC1 RNA (25 μg/500 μl of Opti-MEM) was added to 500 μl of Opti-MEM containing X[1-2,3-diolsoxyloxypropyl]-X,N,N-,trimethylammonium methylsulfate (DOTAP) (50 μg/500 μl of Opti-MEM; Roche, Mannheim, Germany). After 20 min at room temperature, the mixture was added to a 50-ml tube containing 1 x 10^5 DC and incubated at 37°C with occasional agitation for 2.5 h. The DC transfected with MUC1 RNA (DC/MUC1 RNA) and DC transfected with β-galactosidase RNA (DC/β-gal RNA) were washed twice and resuspended in PBS (13). The mature DC/MUC1 RNA were obtained by addition of 0.1 μg/ml LPS (Escherichia coli; Sigma) for 20 h after DC transfected with MUC1 RNA.

Flow cytometry analysis

DC, DC/MUC1 RNA, or DC/β-gal RNA were washed with PBS and incubated with mAb DF3 (anti-MUC1), M1/42/3.9.8 (anti-MHC class I; American Type Culture Collection), M5/114 (anti-MHC class II) Ab was performed on acetone-fixed cells. Lymph nodes were collected from the mice immunized with DC or DC/MUC1 RNA. Frozen sections were prepared on slides were incubated with mAb DF3 for 40 min at room temperature. The slides were washed and incubated with biotinylated horse anti-mouse IgG for an additional 30 min. Red was generated by staining with avidin-biotinylated peroxidase complex solution (Vector Laboratories, Burlingame, CA). The slides were then incubated with MS/114 Ab for 30 min followed by alkaline phosphatase-labeled anti-rat IgG. Alkaline phosphatase/avidin-biotin-peroxidase complex solution (Vector Laboratories) was used to generate a blue counterstain.

Cytotoxicity assay

CTL assays were performed by assessing 51Cr release. Briefly, 1 x 10^6 target cells were labeled for 1 h with 50 μCi of Na25CrO4. Splen cells were obtained from mice immunized with DC, DC/MUC1 RNA, or DC/β-gal RNA. Spleen cells were labeled with mAb DF3 (anti-MUC1) and incubated with mAb DF3 for 20 h after DC transfected with MUC1 RNA, and then analyzed by Student’s t test. Statistical significance was determined at the p < 0.05 level.

Results

Expression of MUC1 on transfected DC

MUC1 and β-gal RNAs were generated by in vitro synthesis (Fig. 1A). Murine bone marrow–generated DC were transfected with the MUC1 RNA or β-gal RNA using the cationic liposome (DOTAP) (13). To assess expression, DC transfected with MUC1 RNA (DC/MUC1 RNA), β-gal RNA (DC/β-gal RNA), and DC alone were examined by immunoblot and flow cytometric analyses. There was no detectable expression of MUC1 in DC transfected with β-gal RNA (Fig. 1B). By contrast, MUC1 expression was detectable in 10–20% DC/MUC1 RNA at 24 h by flow cytometry (Fig. 1B) and immunoblot analysis (Fig. 1C). Transfected DC exhibited a similar cell surface level of MHC class II, B7, and ICAM expression as nontransfected DC (Fig. 1B). Both transfected and nontransfected DC, after stimulation by LPS, increased the expression of MHC classes I and B7-1, and B7-2 (Table 1). β-Gal expression on DC/β-gal RNA was detected by X-gal staining (data not shown).
These findings confirmed the expression of MUC1, MHC classes I and II, B7, and ICAM by DC transfected with MUC1 RNA.

**Function of MUC1-transfected DC**

To determine the function of transfected DC, T cells stimulated with nontransfected DC, DC/MUC1 RNA, or DC/β-gal RNA were compared in MLR assays (Fig. 2A). There were no differences between DC transfected with MUC1 RNA or β-gal RNA and nontransfected DC in terms of stimulating allogeneic and syngeneic T cell proliferation (Fig. 2A). To determine whether the transfected DC migrate to lymph nodes, mice were injected s.c. with transfected or nontransfected DC at the base of the tail. After 24 h, the inguinal lymph nodes were collected and examined by immunohistochemical staining. The lymph nodes from mice immunized with DC/MUC1 RNA exhibited dual expression of MUC1 and MHC class II on cells in T cell areas (Fig. 2B, right). By contrast, MHC class II, but not MUC1, was detectable in lymph nodes from mice injected with control nontransfected DC (Fig. 2B, left). These findings indicate that DC/MUC1 RNA are functional in both T cell activation in vitro and migration to regional lymph nodes in vivo.

### Table I. Comparison of mature and immature DC after transfected with MUC1 RNA

<table>
<thead>
<tr>
<th>Phenotype (mAb)</th>
<th>Nontransfected</th>
<th>Transfected with MUC1 RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immature DC (%)</td>
<td>Mature DC (%)</td>
</tr>
<tr>
<td>DF3/MUC1</td>
<td>0.61</td>
<td>0.84</td>
</tr>
<tr>
<td>MHC I</td>
<td>38.10</td>
<td>41.23</td>
</tr>
<tr>
<td>MHC II</td>
<td>73.83</td>
<td>86.46</td>
</tr>
<tr>
<td>B7.1</td>
<td>55.36</td>
<td>69.15</td>
</tr>
<tr>
<td>B7.2</td>
<td>65.33</td>
<td>97.27</td>
</tr>
<tr>
<td>ICAM</td>
<td>58.05</td>
<td>79.68</td>
</tr>
<tr>
<td>CTL assay&lt;sup&gt;+&lt;/sup&gt; (targets)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type MC38</td>
<td>3.10</td>
<td>1.91</td>
</tr>
<tr>
<td>MC38/MUC1</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>MCF7</td>
<td>ND&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.07</td>
</tr>
<tr>
<td>MUC1.Tg MC38</td>
<td>0.77</td>
<td>1.91</td>
</tr>
<tr>
<td>MUC38/MUC1</td>
<td>0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>MCF7</td>
<td>ND&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Tumor incidence&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MUC1.Tg</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Immature DC were isolated from bone marrow and cultured in 15 ng/ml GM-CSF medium for 7 days; mature DC were cultured with LPS (0.1 μg/ml) for 20 h.

<sup>b</sup> The E:T cell ratio for the 51Cr CTL assay was 30:1. The results of CTLs from mice immunized with mature or immature DC represent the percentage lysis of syngeneic MC38 and MC38/MUC1 (mouse) and MCF7 (human) targets.

<sup>c</sup> ND, Not done.

<sup>d</sup> Tumor incidence was obtained from mice immunized twice with $1 \times 10^6$ mature or immature DC and then challenged with $5 \times 10^5$ MC38/MUC1 tumor cells.
Induction of anti-MUC1 immune responses

To determine whether vaccination with DC/MUC1 RNA induces an anti-MUC1 immune response, wild-type C57BL/6 mice were immunized twice s.c. with $1 \times 10^6$ nontransfected DC, DC/MUC1 RNA, or DC/β-gal RNA. Humoral and CTL responses against MUC1 were evaluated to assess the effectiveness of immunization. Immunization with DC/MUC1 RNA induced the production of anti-MUC1 Abs compared with that found in mice immunized with the nontransfected DC or DC/β-gal RNA (Fig. 3A). Importantly, immunization with DC/MUC1 RNA was also effective in the priming of a CTL response against MC38/MUC1 (Fig. 3B, left), but not MC38 (Fig. 3B, right) and MCF7, targets (Table I). By contrast, splenocytes from the mice immunized with nontransfected DC or DC/β-gal RNA exhibited little if any CTL response against MC38 and MC38/MUC1 cells (Fig. 3B, left and right). Moreover, CTL from the mice immunized with mature DC transfected with MUC1 RNA was higher in lysis of MC38/MUC1 targets than CTL from mice immunized with their immature counterparts (Table I). These results indicate that vaccination with DC/MUC1 RNA induces MHC-restricted, MUC1-specific immune responses.

Induction of antitumor responses

To assess antitumor activity induced by the DC/MUC1 RNA, mice were vaccinated s.c. twice with $1 \times 10^6$ nontransfected DC, DC/MUC1 RNA, or DC/β-gal RNA. The mice were then challenged s.c. with $5 \times 10^5$ MC38 or MC38/MUC1 cells. Vaccination with DC/MUC1 RNA prevented the development of MC38/MUC1 tumors in mice (Fig. 4A and Table II). By contrast, MC38/MUC1 tumor growth was unaffected in the mice immunized with nontransfected DC or DC/β-gal RNA (Fig. 4A). The specificity of these responses against MUC1 is supported by the finding that the vaccinations rendered no protection against the MC38 tumors (Fig. 4B). To assess the effects of DC/MUC1 RNA on treatment of established MUC1-positive tumors, mice were inoculated s.c. with $5 \times 10^5$ MC38/MUC1 or MC38 cells on day 0 and then treated with DC, DC/β-gal, or DC/MUC1 RNA at day 6.
MUC1 RNA, or DC/β-gal RNA on days 3 and 10. Immunization with DC/MUC1 RNA was effective in the elimination of established MC38/MUC1 tumors (Fig. 4C and Table II), but not MC38 tumors (Fig. 4D). By contrast, immunization with DC/β-gal or nontransfected DC had no effect on tumor growth (Fig. 4, C and D, and Table II). These findings demonstrate that immunization with DC/MUC1 RNA is effective in treating MUC1-positive tumors.

Induction of anti-MUC1 immunity in MUC1.Tg mice

Studies have demonstrated that MUC1.Tg mice are unresponsive to stimulation with MUC1 Ag (30, 37). To determine whether vaccination with DC/MUC1 RNA can reverse immunologic unresponsiveness, we immunized MUC1.Tg mice with DC/MUC1 RNA and assessed anti-MUC1 immunity. CTLs from MUC1.Tg mice vaccinated with DC/MUC1 RNA exhibited a low level of MC38/MUC1 cell lysis (Fig. 5A). Moreover, immunization with mature DC/MUC1 RNA failed to increase the CTL activity in MUC1.Tg mice (Table I). By contrast, coadministration of DC/ MUC1 RNA and IL-12 significantly increased the level of CTL activity against MC38/MUC1 cells (Fig. 5A). As a control, there was no detectable CTL activity against MC38 targets (Fig. 5B).

MUC1.Tg mice immunized with DC/MUC1 RNA also exhibited no apparent protection against challenge with MC38/MUC1 cells (Fig. 5C and Table II). However, coadministration of DC/MUC1 RNA and IL-12 to the MUC1.Tg mice was associated with rejection of MC38/MUC1 (Table II), but not MC38, tumor cells (Fig. 5, C and D). By contrast, there was no rejection of either MC38/ MUC1 or MC38 tumors by MUC1.Tg mice immunized with DC or DC/β-gal RNA (Fig. 5, C and D). These results indicate that the immune response induced by immunization of MUC1.Tg mice with DC/MUC1 RNA is insufficient to prevent MUC1-positive tumor cell growth and that IL-12 is required to reverse immunologic unresponsiveness to MUC1 in these mice.

Discussion

The induction of immunity against the human MUC1 Ag has been studied in animal models and clinical trials of active specific immunotherapy (23). MUC1-based vaccines have been shown to induce humoral responses; however, activation of a cellular response has been judged to be insufficient to be effective against MUC1-positive tumors (29, 38–40). Recent work has shown that vaccination of mice with recombinant vaccinia viruses expressing

Table II. Antitumor immunity induced by DC transfected with MUC1 RNA

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Tumor Challenges (5 × 10^5)</th>
<th>Wild-Type</th>
<th>MUC1.Tg prevention</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>MC38/MUC1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DC</td>
<td>MC38/MUC1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DC/β-gal</td>
<td>MC38/MUC1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DC/MUC1</td>
<td>MC38/MUC1</td>
<td>0^a</td>
<td>0^a</td>
</tr>
<tr>
<td>IL-12</td>
<td>MC38/MUC1</td>
<td>ND^c</td>
<td>ND</td>
</tr>
<tr>
<td>DC/MUC1 + IL-12</td>
<td>MC38/MUC1</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

^a Tumor incidence was determined on day 10. Tumor size ≥3 mm was considered positive.
^c All mice were tumor free on day 10 but one mouse developed tumor on day 20.
^d ND, Not done.
MUC1 and the B7 costimulatory molecule induces potent anti-MUC1 immunity (34). DC transduced with viral vectors that express MUC1 also induce antitumor activity against MUC1-positive carcinomas in mice (41, 42). Similar findings have been obtained with vaccines derived from the fusion of DC with MUC1-positive tumor cells (31, 43).

Induction of antitumor immunity with tumor RNA-transfected DC represents another approach for DC-based vaccines (17, 18). RNA preparations for transfection of DC can be transcribed from cDNA (13) or can be extracted from tumor cells (17, 18). In the present studies, DC were transfected with MUC1 RNA. The transfected DC expressed MUC1 Ag, costimulatory, and adhesion molecules. Immunization with MUC1 RNA-transfected DC was associated with induction of an MUC1-specific CTL response and resulted in the rejection of MC38/MUC1, but not MC38, tumors in wild-type mice. By contrast, there was no evidence for induction of anti-MUC1 immunity in mice immunized with nontransfected or β-gal RNA-transfected DC. These findings indicate that vaccination with MUC1 RNA-transfected DC is effective in inducing specific anti-MUC1 immunity in vivo.

C57BL/6 mice transgenic for human MUC1 (MUC1.Tg) have been developed to study immunologic responsiveness to the human MUC1 tumor-associated Ag (30). Immunizations with purified human MUC1 Ag and irradiated MUC1-positive (MC38/MUC1) tumor cells were associated with induction of an anti-MUC1 Ab response and no detectable cytotoxic T cell reactivity (44). Recent work has demonstrated that DC play a pivotal role in the regulation of immunity and tolerance (45–48). In our system, DC/MUC1 RNA activated by LPS up-regulated MHC class I and II and costimulatory molecules and increased CTL activity in the wild-type mice, but not in the MUC1.Tg mice. The findings that immunization with MUC1 RNA-transfected DC was effective against challenge of MUC1-positive tumors in the wild-type mice, but failed in MUC1.Tg mice, indicated the existence of tolerance to MUC1, even immunization with mature DC/MUC1 RNA. However, coadministration of IL-12 prevented the tolerance induction.

The induction of tolerance has been reported using peptide-pulsed DC under ineffective priming conditions, including neutralization of endogenous IL-12 (49, 50). IL-12, a heterodimeric cytokine, has been found to induce stimulation of Thl reactivity and enhancement of CTL responses (51, 52). Moreover, IL-12 has been demonstrated to up-regulate DC expression of the B7 molecule (53), inhibit anergy induction, and reverse unresponsiveness to tumor peptides (54). Coadministration of IL-12 resulted in strong proliferative response of CD8+ T cells and development of lytic effector function (55, 56). The mechanism of IL-12 activity in reversal of immunologic unresponsiveness in vivo is still unclear (54). Recent studies indicated that IL-12 acted as a third signal, along with TCR and costimulatory molecules, to reverse Ag-induced tolerance and expand Ag-specific CD8+ T cells (55, 56). In the present studies tolerance to MUC1 was reversed by coadministration of the MUC1 RNA-transfected DC and IL-12. It is possible that IL-12 functions as adjuvant to provide an optimal environment for the induction of antitumor immunity in MUC1.Tg mice (57). Our findings demonstrate that immunologic unresponsiveness to MUC1 in the MUC1.Tg is reversible when DC present MUC1 epitopes in the presence of potentiating signals induced by IL-12.

References


