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Global Gene Expression Profiling in Interleukin-12-Induced Activation of CD8⁺ Cytotoxic T Lymphocytes against Mouse Mammary Carcinoma

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Abstract

Interleukin-12 (IL-12) is a critical cytokine representing the link between the cellular and humoral branches of host immune defense apparatus. IL-12-induced cytotoxic lymphocyte (CTL) development is a central mechanism in immune responses against intracellular infectious agents as well as malignant growth. However, the molecular basis of tumor-specific CTL responses mediated by IL-12 remains poorly defined. In this study, we addressed this issue in a comprehensive manner to probe into IL-12-induced anti-tumor responses by global gene expression profiling of mRNA expression in CD8$^+$T cells in a transplantable syngeneic mouse mammary carcinoma model treated or not with recombinant IL-12. A strong tumor regression was induced by the IL-12 treatment. An introspection of differential gene expression at an early stage of the IL-12-initiated CTL activation reveals interesting genes and molecular pathways that may account for the marked tumor regression, and is likely to provide a rich source of potential targets for further research and development of effective therapeutic modalities.

Keywords

interleukin-12; cytotoxic T lymphocyte; TS/A; mammary carcinoma; microarray

Introduction

The emergence of the concept of cancer immunosurveillance conceived by Paul Ehrlich at the beginning of 20th century and formalized by Burnet and Thomas in 1957, the severe challenges it faced in the 1970s and 80s (1–5), and its renaissance in the last decade brought about by a large body of mouse (6–11) and human (12–17) studies in immunodeficient settings have helped solidify the belief that components of the immune system, such as lymphocytes (T, NK, NKT) and cytokines (IFN-γ, perforin, IL-12) are critically involved in controlling primary tumor development. The refinement of this concept and its extension to “immunoediting” has been put forward by R.D. Schreiber and colleagues to describe the dual host-protecting and tumor-sculpting actions of the immune system that not only impede but also mark neoplastic alterations for immune elimination (18,19).

Tumors often possess a number of potential recognition sites for immunologic effector cells, which, in theory, could make them susceptible to immune surveillance. Nevertheless, most of such tumors grow progressively in their natural hosts or syngeneic recipients, without being controlled effectively by the immune system. The lack of apparent immunogenicity of tumors

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in situ might be due to special properties of the tumor cells, e.g., lack of costimulatory molecules, down-regulation of MHC molecules, or production of immunosuppressive factors (20,21), or due to intrinsic tolerance mechanisms of the immune system (22).

The immune response against cancer cell is complex, involving the interaction of many different cell types and cellular products. However, it is well established cytotoxic T lymphocytes (CTLs) constitute one of the most important anti-tumor immunity (23,24). CTLs recognize, *via* their clonal T cell receptors, specific antigenic peptides that are derived from tumors, especially solid ones including carcinomas and sarcomas (25,26), and presented to them by antigen-presenting cells such as dendritic cells (DCs). These CTLs survey the body and lyse malignant cells expressing the relevant surface antigens. This mechanism of recognition and killing is analogous in many ways to viral infections of peripheral tissues for which CTL is the main effector of adaptive immunity controlling the spread of the infection (27).

In recent years, immunotherapies have been rekindled that attempt to either mark the tumor by upregulating the surface antigens for enhanced interaction with immune effector cells (21, 28), or by directly activating DC, T and NK cells for their heightened “scouting” capacity and increased cytolytic potency. IL-12 is a factor belonging to the latter class (29). IL-12 is a pivotal cytokine representing the link between the cellular and humoral branches of host immune defense apparatus. IL-12 is a heterodimer produced primarily by macrophages and DCs in both innate and adaptive immune responses. It is a key factor in the induction of T cell-dependent and independent activation of macrophages, NK cells, generation of T helper type 1 (Th1) cells and CTLs, induction of opsonizing and complement-fixing antibodies, and resistance to intracellular infections (30). IL-12 has powerful anti-tumor and anti-metastatic activities against many murine tumors as well as human tumors (31). The potent anti-tumor activities of IL-12 are mediated through similar mechanisms that are used by IL-12 against infectious agents, i.e., *via* the activation of NK cells for the bulk of the non-antigen specific clearance, and activation of CTL and CD4 for tumor-specific elimination and long-term immunity. Numerous recent studies across a wide range of experimental tumor model systems as well as in human cancers have unequivocally confirmed the efficacy of IL-12 as a potent inducer of CTL-mediated anti-tumor immunity (32–47), thus strongly justifying further exploration of IL-12 and CTL in cancer immunotherapy. Furthermore, in a recent study, Lee et al. reported for the first time that IL-12 could inhibit activation-induced CD8$^+$T cell death by downregulating Fas ligand and up-regulating cellular FLIPs, followed by suppressing activation of caspases 8 and 3, thus providing a survival signal for sustained CTL responses (48).

TS/A is an aggressive and poorly immunogenic cell line established from a moderately differentiated mammary adenocarcinoma that arose spontaneously in a multiparous BALB/c mouse (49). It grows progressively, kills both nu/nu and syngeneic mice, and gives rise to lung metastases. It expresses MHC class I (H-2D^d, H-2K^d), but not class II molecules, secretes G-CSF, GM-CSF, TGF-β, basic fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), and does not stimulate a syngeneic antitumor response *in vivo*, nor in mixed lymphocyte-tumor cell cultures (50). IL-12 administered to TS/A tumor-bearing mice *via* various routes at different stages of tumor establishment has been shown to cause the rejection of the tumor in wild type BALB/c mice through a CD8$^+$T-lymphocyte-dependent reaction associated with macrophage infiltration, vessel damage, and necrosis. Protective immune memory is also established following IL-12 treatment (51).

Genome-wide gene expression profiling allows the viewing, with molecular accuracy, of biological processes as a whole. The mRNA expression of tens of thousand of genes in response to a given signal can be monitored simultaneously using massively parallel DNA microarray technology. The vast amount of data generated can be organized, refined and extrapolated with the aid of bioinformatics tools to reveal a comprehensive, highly concerted, and logical molecular program underlying the process of cellular differentiation and activation (52,53). Coordinate gene expression takes place among products of the genes that function in a common differentiation program or in the same physiological response pathway. Recent applications of this powerful technology to the analysis of the immune system have uncovered many potential novel pathways and key players in normal as well as pathological immune responses, which are likely to have a great impact on our understanding of autoimmunity, immune deficiencies, and cancers of immune cells (54–58).

The aim of this study was to obtain a high resolution, dynamic signature of a tumor-specific CTL response at every step during its development after activation in lymphoid tissues. The approach was to use microarray to monitor gene expression in the CD8$^+$T cell compartment in the spleen of mice given TS/A tumor cells and recombinant IL-12 over a period of four weeks spanning primary activation, effector phase and memory formation. The choice of TS/A for this study was based on three considerations: (i) these tumor cells expresses MHC class I; (ii) immunotherapy with GM-CSF and IL-2 has demonstrated a strong tumor-specific CTL response and protective immunity (59); (iii) IL-12 administration is effective against these tumors by a CD8$^+$T- or NK-dependent mechanism. Therefore, the TS/A model is highly relevant to studying tumor-specific CTL responses regulated by IL-12. We performed Affymetrix microarrays using RNA samples isolated from purified splenic CD8$^+$T cells of TS/A tumor-bearing mice.

Materials and Methods

Mice

Female BALB/c mice (6–8 week old) were purchased from the Jackson Laboratory (Bar Harbor, Maine). All mice were housed at the Weill Medical College of Cornell University Animal Facilities in accordance with the Principles of Animal Care (NIH publication no. 85-23, revised 1985). Mice bearing TS/A tumors were all sacrificed no later than Day 35 due to the morbidity caused by large tumor size and strong metastasis.

Tumor implantation, size measurement

TS/A mammary carcinoma cells (1×10^5) were injected subcutaneously into the abdominal mammary gland area of recipient mice in 0.1 ml of a single-cell suspension in phosphate buffered saline (PBS) on Day 0. The dose of tumor implantation was empirically determined to give rise to tumors of ~10 mm in diameter in untreated wild type mice in 28 days. Primary tumors were measured using electronic calipers every 3–4 days. Reported measurements are the square root of the product of two perpendicular diameters.

IL-12 treatment

Recombinant murine IL-12 was provided by Genetics Institute (Cambridge, Massachusetts). IL-12 treatment was given by intraperitoneal injection at 1 μg per mouse every other day starting on Day 7 until the end of each experiment unless other wise described. This regimen of IL-12 was well tolerated with no signs of toxicity.

Purification of splenic CD8$^+$T lymphocytes

BALB/c mice spleen cells were prepared by using 40 μm cell strainer (BD Falcon, Bedford, MA), and the red blood cells were lysed with ACK buffer (60), and then washed with RPMI 1640 media with 10% FBS three times. The cells were resuspended at a concentration of 2×10^7/ml in cold PRMI 1640/10% FBS media, and then Dynabeads (CELLection mouse CD8 kit, DYNAL, Lake Success, NY), were added (25 μl containing 10^7 beads/2 × 10^7 spleen cells) using the magnetic particle concentrator (Dynal MPC) to positively select the mouse spleen CD8$^+$T cells. Following capturing of the bead-linked CDS8$^+$T cells, the bead-releasing step was omitted before RNA isolation.

Microarray experiment

The high-density oligonucleotide microarray system of Affymetrix (Santa Clara, California), murine Genome U74A Array version 2 containing 12,488 genes, was used. Total RNA was isolated from freshly isolated spleens of all surviving mice on Day 7 (before IL-12 treatment), and Day 14 (one week following the initial IL-12 injection). RNA samples of each mouse within each experiment group were pooled from 3–4 mice. 10 μg total RNA was used to synthesize cDNA using Superscript cDNA synthesis kit (Invitrogen, Carlsbad, California) with a primer containing oligo (dT) and T7 RNA polymerase promoter sequences. Double-stranded cDNA was then purified by phase lock gel (Eppendorf, Westbury, NY) with phenol/chloroform extraction. The purified cDNA was used as a template to generate biotinylated cRNA using the Bioarray High Yield RNA Transcript Labeling Kit (Enzo Biochem), and then biotinylated cRNAs were fragmented and hybridized to Affymetrix Test 3 chips (Affymetrix Inc., Santa Clara, CA). All RNA samples passed quality control (ratio of 3′ to 5′ < 3), then the samples were hybridized to the Murine Genome Array U74Av2 array which contains 12,488 well-substantiated mouse genes. After overnight hybridization, the arrays were washed, stained with streptavidin-phycoerythrin (Molecular Probes, Eugene, OR) on the GeneChip Fluidics Station (Affymetrix), and scanned according to the standard Affymetrix protocol.

Microarray Data collection and analysis

Affymetrix GeneChip 5.0 was used as the image acquisition software for the U74Av2 chips. The signal, which represents the intensity of each gene, was extracted from the image. The target intensity value from each chip was scaled to 250. Data normalization, log transformation, and statistical analysis were performed with GeneSpring software (Silicon Genetics, Redwood City, CA). Array data were globally normalized in two steps. Firstly, all of the measurements on each chip were divided by the 50th percentile value (per-chip normalization). Secondly, each gene was normalized to the baseline value of the control samples (per-gene normalization).

Statistical tests

Tumor growth data to be compared were first subjected to normality test. Where the samples studied were normally distributed, statistical comparisons were performed using the Students' *t* test. Where the samples deviated from normality, a nonparametric, Mann Whitney Rank-Sum test was used for comparisons. Statistical analyses were performed using SigmaStat software. For all experiments, the mean and the SD are depicted.

Results and Discussion

IL-12 induces anti-tumor activities in vivo

To assess the effects of IL-12 in tumor regression, TS/A mammary carcinoma was initiated by s.c. injection of TS/A cells into mice on the syngeneic BALB/c background. Recombinant mouse IL-12 (rmIL-12) was given i.p. starting on Day 7 post tumor injection to mice when the primary tumor had grown to ~ 4–5 mm in diameter. The timing of IL-12 administration was based on potential therapeutic considerations to mimic clinical situations in which breast cancer patients do not get therapy until the presence of malignant growth in the breast has been identified by mammogram or other means. As shown in Figure 1, by Day 35 (four weeks following the initial IL-12 injection), there was a strong regression in the TS/A tumor growth in mice given IL-12 treatment. The results are consistent with published data (51) showing great efficacy of IL-12 as a therapeutic agent in this model.

Genome-wide analysis of gene expression in splenic CD8$^+$ lymphocytes

Our main interest in this study was to investigate the molecular mechanism(s) by which IL-12 activates CTL against the developing TS/A tumor. The strategy we chose to achieve this objective was to use DNA microarray to comprehensively survey the gene expression in the CD8$^+$T cell populations in the spleen of mice treated or not with IL-12 over the entire phase of tumor growth or regression, with the hope to identify molecular targets that may play critical roles in mediating IL-12's anti-tumor activity by activating CTL. As a first step towards obtaining a detailed description of the molecular events taking place in the CD8$^+$ T cells in mice treated or not with rmIL-12, we performed global gene expression analysis of the two groups of tumors on Day 14 (7 days following the initial IL-12 injection) using the Affymetrix oligonucleotide microarray system (Murine Genome U74A Array version 2 containing 12,488 genes). To reduce variations between individual mice, RNA samples were pooled from all mice within each group for the microarray analysis. We applied this technology to the search of genes that undergo altered expression in CD8$^+$ splenic T cells *in vivo* following IL-12 therapy in an attempt to identify the downstream targets of IL-12 in this particular lymphocyte compartment. RNA samples were prepared from the two experimental groups shown in Figure 1 for comparison of differential gene expression. This microarray experiment (4–11 mice from each group) yielded a large amount of data, which was processed through the GeneSpring software for (i) data normalization (bias correction), (ii) data transformation (to ensure normal distribution), and (iii) gene filtering to identify specific genes that are expressed differentially by using appropriate statistic tools. The group of PBS-treated wild type mice was set as the baseline (with an expression value of 1.0) to which the IL-12-treated group was compared. Most of the genes show no altered expression. The vast majority of the genes were either present equally or absent in both samples. A small number of genes manifested changes in expression to varying degrees. These are summarized in Table 1a and 1b as IL-12-induced genes and IL-12-inhibited genes, respectively.

Although the CD8$^+$T cell response activated by IL-12 analyzed in this study was a total one instead of being specific for given target molecules in the TS/A tumor because of the lack of identified tumor antigens, interesting information regarding the CTL response to a developing tumor can still be gleaned and extrapolated from the microarray data. On the side of the induced genes by IL-12, many of them were quite as expected. Genes that are involved in proteolysis, cytotoxic killing, and cell migration were found, e.g., several types of carboxypeptidases, myeloperoxidase, proteinase 3, neutrophil elastase, caspase-1 (IL-1-converting enzyme, ICE), NK killer receptor NKG2-D, Ly49b, IFN-γ-induced GTPases and chemokines (IP-10, macrophage inflammatory protein MIP), chemokine receptors (CCR1 and CCR5), etc.

One surprising finding is the strong induction of IL-6 by IL-12. IL-6 is a multifunctional cytokine that regulates cell growth, differentiation, and cell survival in the immune system. These two cytokines are not known to interact and cross-talk. In some malignant tumors, tumor-infiltrating lymphocytes (TIL) secrete IL-6 (61,62). IL-6 completely antagonizes the immunosuppressive effects of TGF-β1 on T cell proliferation in eyes with endotoxin-induced uveitis (63). Combined IL-2 and IL-6 gene therapy, by liposome-mediated intratumor delivery to mice bearing B16F10 melanoma, significantly enhances the CTL and NK activity of splenocytes and TILs (64). TIL secretion of IL-6 antagonizes tumor-derived TGF-β1 and restores the lymphokine-activated killing activity against canine transmissible venereal tumor (CTVT) (65). In light of these new findings and our own data, the role of IL-6 in tumor-specific CTL responses and the interaction between IL-12 and IL-6 should be explored.

A much less appreciated aspect of IL-12 immunobiology is the flip side of the coin, i.e., its inhibitory activities. Among the genes that were inhibited by IL-12 in the CD8$^+$T cells are more novelties. Take for example, guanosine diphosphate (GDP) dissociation inhibitor 2 (GDI-2). The GDP dissociation inhibitors (GDIs) represent an important class of regulatory proteins in the functional cycle and recycling of Rab GTPases. Accumulated evidence over the past several years points to a functional role for the Rab/YPT1/SEC4 gene subfamily of p21 ras-like small GTP-binding proteins in the mechanisms regulating membrane trafficking in yeast and mammalian cells. Reported data are consistent with the notion that Rabs are essential in each step of vesicular transport, including vesicle formation, vesicle docking, and membrane fusion (66). Among the three presently known members of the GDI protein class (GDI-1, also known as RabGDI or GDIα, GDI-2, and GDIβ) (67–70), GDI-1, the best-studied member, appears crucial for progression through the membrane/cytosol localization cycle and the recycling of all Rabs. Because GDIs display a high degree of homology (86% and 96% identity between GDI-2 and GDI-1, or GDIβ, respectively), it is largely accepted, yet not proven, that they exhibit a redundant function. Although several lines of biochemical and morphological evidence suggest distinct functional roles for GDI-1 and GDI-2 in the context of living cells (71–73), no specificity toward an individual Rab or a subset of Rab proteins has been documented. Thus, GDI-2 displays a general activity to release Rabs from membranes (74). Although the involvement of Rabs in cancer is presently unknown, their regulation by GDIs could be an interesting direction in immune response to developing cancer.

Polyomavirus enhancer activator 3 (PEA3) is the prototypical member of one major subgroup of the superfamily of Ets-related transcription factors which, at the present time, is composed of three members, *PEA3* (also known as *E1AF* or *ETV4*) (75), *ER81*/*ETV1* (76) and *ERM*/*ETV5* (77). These three transcription factors are > 95% identical in the Ets-domain and > 85% in the transactivation acidic domain (78). Several candidate target genes of the PEA3 transcription factor have been reported based largely on the occurrence of Ets-binding sites in their upstream regulatory regions. A large proportion of these genes encode matrix proteases (MMP-2, -7, -9, -13, -14 and -19, uPA) and some of their inhibitors (TIMP-1), whose dysregulated expression has been associated with the invasive potential of tumor cells (79–84). Expression of PEA3 has been positively linked to MMP2, NRG1 and CGB expression in breast tumorigenesis (85). It would be interesting to establish the role of PEA3 in IL-12-mediated anti-tumor CTL response.

IL-12-induced CTL development is a central theme in the immune responses against intracellular infectious agents as well as against malignant growth, united by a common mechanism regulated by IL-12. The microarray data collected at various stages of a tumor-specific CTL development induced by IL-12 represent a comprehensive molecular survey of these important differentiation events in a biologically dynamic and kinetic manner. With the aid of sophisticated bioinformatic tools and experimental verification, we will be able to uncover previously unknown pathways and identify potential targets critical for CTL activation in response to tumor development. These novel pathways and targets will be invaluable to our efforts to understand the process of anti-tumor responses effected by the immune system.

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Abbreviation

CTL	cytotoxic T lymphocyte
DC	dendritic cell

FGF	fibroblast growth factor
VEGF	vascular endothelial growth factor
GDP	guanosine diphosphate
CTVT	canine transmissible venereal tumor
PEA3	polyomavirus enhancer activator 3
MIP	macrophage inflammatory protein

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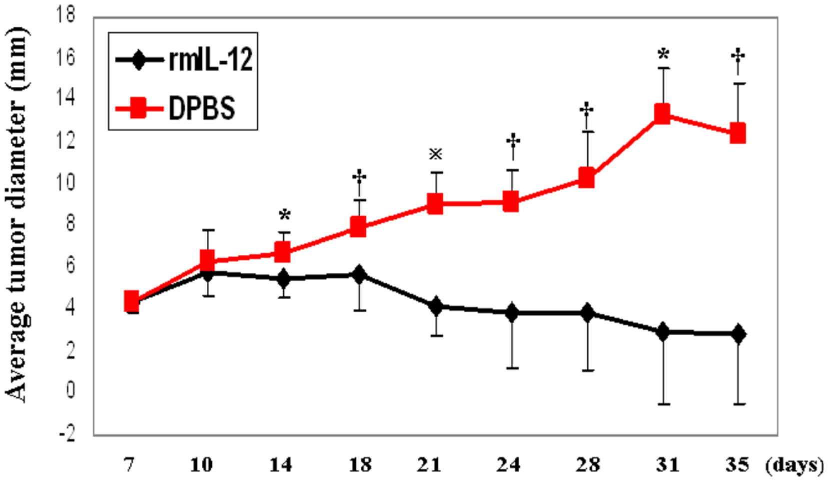

Figure 1.

TS/A tumor growth in syngeneic mice. TS/A cells were injected subcutaneously in the middle of the right flank of BALB/c mice with 0.1 ml of a single-cell suspension containing the indicated number of TS/A cells. Tumor growth was monitored every 3–4 days and size measured with a caliper. Each data point is comprised of 4–11 mice. Error bars represent standard deviation. *$p < 0.05$; †$p < 0.01$; ※$p < 0.0001$.

Table 1

Genes differentially regulated by IL-12. Genes are selected based on the following criteria: (1) They must be expressed more than 2 times than the respective control sample (PBS-treated) to qualify as IL-12-induced genes (Table 1a), or 2 times less than the control to qualify as IL-12-inhibited genes (Table 1b); (2) Their expression detection must be statistically valid as present with detection $p < 0.05$; Genbank accession numbers are given in the second column. The numbers in column 3 are relative fold of expression over the control (PBS-treated group).

Table 1a. ***Genes induced by IL-12 (117 genes).***

Gene and description	Genbank accession	Fold induction	Gene and description	Genbank accession	Fold induction
Aldehyde dehydrogenase (ALDH2)	U07235	2.01	Ig rearranged k-chain mRNA, clone AN08K.	M19911	4.35
Aldehyde dehydrogenase 2, mitochondrial	AI647493	2.24	Ig variable light chain.	X88903	2.05
Alpha fetoprotein	AV037200	3.03	Ig Vkappa-HNK20	X82688	3.43
AMY, Noe1, OlfA, Pancortin, Pancortin3	D78265	4.86	IgA	J00475	18.97
Annexin A2	M14044	2.2	IgA	J00475	3
Annexin A3	AJ001633	2.34	IgA V-D-J-heavy chain	X94418	4.2
Anti-DNA light chain IgM, antibody 363p.168	U55576	3.1	IgG variable region.	Z22111	2.53
Antigen identified by monoclonal antibody Ki 67	X82786	2.23	Igh	AF042086	2.65
Atf3, leucine zipper transcription factor LRG-21	U19118	3.79	Igh-6	X94422	2.93
Balb/c neutrophil elastase gene, exons 4 and 5	U04962	3.1	Igk-V28	U62386	3.63
Bmk, Hck-1	J03023	2.82	IL-1receptor antagonist	L32838	3.13
Carbonic anhydrase I (CAI)	M32452	23.21	Immediate early response 3	X67644	2.62
Carboxypeptidase A3, mast cell	AV172041	10.9	Immunoglobulin heavy chain variable gene	X16740	5.85
CB17 SCID immunoglobulin heavy chain V region	U23095	2.43	Immunoglobulin kappa chain variable 28 (V28)	Z70661	3.68
CD24a antigen	M58661	2.81	Immunoglobulin kappa chain variable 28 (V28)	U48716	3.49
CDR3 region; Ig heavy chain gene	AF042798	3.77	Immunoglobulin superfamily, member 4	AF061260	2.78
Cell division cycle 25 homolog C (S. cerevisiae)	L16926	3.56	Immunoglobulin superfamily, member 4	AF061260	2.03
c-Fes	X12616	2.14	Interferon inducible protein 10 (IP-10)	M33266	5.18
Chemokine (C-C) receptor 1	U29678	2.36	Interferon-inducible GTPase	AA914345	5.26
Chemokine (C-C) receptor 5	AV370035	2.22	Interferon-inducible GTPase	AJ007971	4.28
Chitinase 3-like 3	M94584	2.17	Interleukin 1 beta	M15131	2.21
Complement component 4 (within H-2S)	X06454	5.44	Interleukin 6 (IL-6)	X54542	20.27
Cpa3, carboxypeptidase A	J05118	7.37	Killer cell lectin-like receptor, Ly49b	U10304	2.77
Ctla2b	X15592	4.45	Kinesin-like 1	AJ223293	2.88
Ctsg, serine proteinase	X70057	14.48	Lipo 1, lipocortin I	M69260	3.3

Table 1a. ***Genes induced by IL-12 (117 genes).***

Gene and description	Genbank accession	Fold induction	Gene and description	Genbank accession	Fold induction
C-type lectin, superfamily member 6	AJ133533	2.55	Lipoprotein lipase	AA726364	5.86
Cx26, connexin	M81445	2.77	Ly-6G.1	X70920	7.16
Emr1, F4/80	X93328	11.56	Mitotic checkpoint protein kinase (Bub1)	AF002823	2.26
Entpd1, ecto-apyrase CD39	AF037366	2.91	Myeloperoxidase	X15313	9.08
Epcr, endothelial cell activated protein C	L39017	2.58	N-acylsphingosine amidohydrolase 1	AW124297	2.14
Epstein-Barr virus induced gene 3	AF013114	2.14	Nfe2, basic leucine zipper transcription factor	L09600	2.65
EST	AI317217	12.66	NKG2-D (Nkg2d)	AF054819	3.31
EST	X67210	6.95	NP-1, neuropilin	D50086	2.87
EST	X67210	5.7	paraoxonase-3 (Pon3)	L76193	2.32
EST	AL078630	5.51	PLA2, Calcium-dependent phospholipids binding protein	M72394	2.79
EST	AW259499	3.3	Plasma glutamate carboxypeptidase	AF009513	3.84
EST	AI854793	3.16	precursor of C and V-D-J regions from 7B6.8	D14625	3.44
EST	AI842940	2.98	Primary response gene B94	L24118	9.04
EST	AW215456	2.65	Proteinase 3, myeloblastin	U43525	6.34
EST	AI841689	2.42	Ptpn13, protein tyrosine phosphatase	D83966	17.04
EST	AW123773	2.29	PYT, MPS1L1, putative esk kinase	M86377	4.04
EST	AA590345	2.17	Ra175c	AB021966	2.2
EST, similar to 202 interferon-activatable protein	AV229143	4.11	Rab6, kinesin-like	Y09632	2.3
EST, similar to gb:M33308 vinculin (human)	AI462105	2.43	rhom-2	M64360	2.88
EST, similar to gb:X52634 tlm oncogene	AI504305	4.16	S100 calcium binding protein A1	AF087687	2.84
Fc receptor, IgE, high affinity I	J05018	4.24	Secreted phosphoprotein 1	X13986	5.6
Fcgr1	M31314	3.89	Sid23	AB025406	2.08
Formyl peptide receptor, related sequence 2	AF071180	6.03	Small inducible cytokine A3	J04491	3.61
germline immunoglobulin V(H)II gene H18	X02468	2.27	Spi-1, PU.1	L03215	2.27
Hex (Prh)	AB017132	2.07	spi2/eb1	M64085	3.38
Histidine decarboxylase cluster	X57437	2.79	ST2L	D13695	6.63
ICE, Il1bc, Caspase-1	L28095	2.4	Stx3, syntaxin 3A	D29797	3.42
Ier5, immediate early response 5 gene	AF079528	3.94	Stx3, syntaxin 3D-2	D38375	15.65
Ifi202, Ifbip-1	M31418	4.07	TCRγ-V4	X00697	13.42
Ig B cell antigen receptor gene	L28059	7.61	Tiap, IAP repeat	AB013819	2.08
Ig B cell antigen receptor gene	L28060	4.06	UPase, UdRPase, uridine phosphorylase	D44464	11.84

Table 1a. ***Genes induced by IL-12 (117 genes).***

Gene and description	Genbank accession	Fold induction	Gene and description	Genbank accession	Fold induction
Ig g-3 V-D-J region	D14625	5.47	Vh186.2/Jh2	AF065324	2.98
Ig heavy chain 6 (heavy chain of IgM)	X94420	4.07	Vk10c	AF029261	3
Ig heavy chain variable region precursor, gene	AF036737	3.99			

Table 1b. ***IL-12-inhibited genes (169 genes).***

Gene and description	Genbank accession	Fold inhibition	Gene and description	Genbank accession	Fold inhibition
ALL 1-fused gene from chromosome 1q	U95498	2.93	f8a, factor VIII-associated protein	M83118	2.5
Angiotensin converting enzyme	J04946	2.07	Fatty acid amide hydrolase gene	AF098009	2.18
Antigenic determinant of rec-A protein	X58472	2.01	Fc receptor, IgG, alpha chain transporter	L17022	5.59
Arp1, alpha-Arp1	AB010297	2.12	Feminization 1 homolog a (C. elegans)	AI836048	2.07
ATPase, H+ transporting, lysosomal (vacuolar proton pump), subunit 1	AI646638	3.21	FK506 binding protein 4 (59 kDa)	X17069	2.2
BAP, Bap37	AC002397	2.02	galK, galactokinase	AB027012	2.26
Bat-4	L76155	2.14	General control of amino acid synthesis-like 2 (yeast)	AW049299	2.07
Bromodomain-containing 4	AI838366	2.44	Glns-ps1, outative introless glutamine synthetase	M60803	2.55
BSP1, Nrpn, mGk-8, Prss19, TADG14	D30785	2.24	Gna11, guanine nucleotide binding protein	U37413	2.83
Bystin-like	AI132491	2.04	GR, glucagon receptor	L38613	2.08
Cannabinoid receptor 2 (macrophage)	X86405	2.43	Granzyme G	J02872	2.04
Casein kinase II, alpha 1 related sequence 4	U51866	2.23	Guanosine diphosphate (GDP) dissociation inhibitor 2	U07951	5.16
Cd28	M34563	2.01	Histocompatibility 2, L region	AI326621	3.13
CD3 antigen, delta polypeptide	X02339	2.01	Histone cell cycle regulation defective homolog A (S. cerevisiae)	AW125193	2.72
CD6 antigen	U12434	2.09	Hnrpa1, alternative splicing modulator	U65316	3.15
Cerebellar degeneration-related 2	U88588	2.99	hsp40	AB028272	2.76
Chaperonin subunit 5 (epsilon)	AV170770	2.24	hsp-E7I	L40406	2.8
Chemokine (C-X-C) receptor 4	Z80112	2.42	IGFBP-4, insulin like growth factor binding protein 4	X76066	2.1
Chromobox homolog 1 (Drosophila HP1 beta)	X56690	20.49	IL-4 receptor (IL4R)	M27960	2.21
CIS, cytokine SH2-containing protein	D89613	2.25	IL-7 receptor (IL7R)	M29697	2.66
Cleavage and polyadenylation specificity factor 3	AI849311	2.56	Insulin-like growth factor I receptor	AF056187	2.09
Cmah, CMP-N-acetylneuraminic acid hydroxylase	D21826	2.02	Intercellular adhesion molecule 2	X65493	2.4
Ctps2, CTP synthetase homolog	U49385	2.29	K^+ intermediate/small conductance Ca^{2+}-activated channel, subfamily N, member 4	AF042487	2.36

Table 1b. ***IL-12-inhibited genes (169 genes).***

Gene and description	Genbank accession	Fold inhibition	Gene and description	Genbank accession	Fold inhibition
CYL2, D-type cyclin	M83749	2.5	Kallikrein 8	AV145185	2.42
Defender against cell death 1	AV099898	2.16	Keratin complex 1, acidic, gene 10	V00830	3.38
DNA polymerase epsilon, subunit 2	AF036898	2.21	Kruppel-like factor 2 (LKLF)	U25096	2.3
DNA segment, Chr 2, ERATO Doi 391, expressed	AW121160	2.15	LEF-1	D16503	2.29
DnaJ (Hsp40) homolog, subfamily B, member 10	AI843164	2.63	LEF-1	D16503	2.02
EBI 1, G-protein coupled receptor	L31580	2.13	Leucine zipper-EF-hand containing transmembrane protein 1	AI851685	2.62
Epoxide hydrolase 1, microsomal	U89491	2.52	lpC1, G protein-coupled receptor EDG6	AJ006074	2.28
EST	AV356018	6.02	Lymphocyte protein tyrosine kinase	AV314529	2.1
EST	AA673252	4.96	Max dimerization protein 4	U32395	2.49
EST	AW061161	2.76	Melanocyte proliferating gene 1	AI842612	2
EST	AI843106	2.48	MEN1, menin	AB023401	2.36
EST	AI854141	2.46	Mesoderm development candiate 2	AW045534	2.38
EST	AW011716	2.44	Mitochondrial ribosomal protein L53	AI854607	2.09
EST	AV274270	2.44	MMET-1, granzyme M	AB015728	2.07
EST	AV164757	2.44	Mouse ORF	M37030	2.49
EST	AV117844	2.43	mRECK, metastasis and invasion	AB006960	2.39
EST	AW259411	2.42	Ndr2	AB033921	3.19
EST	AI842128	2.4	Npm3	U64450	2.08
EST	AI834777	2.38	P4ha1, prolyl 4-hydroxylase alpha(I)-subunit	U16162	2.36
EST	AI846549	2.38	PAC1, tyrosine-threonine dual specificity phosphatase	U09268	2.01
EST	AA795923	2.36	Period homolog (Drosophila)	AF022992	2.05
EST	AW049142	2.36	Pkcz, protein kinase C zeta	M94632	2.61
EST	AW050018	2.33	Polymerase, gamma	U53584	2.05
EST	AV352777	2.33	Polyomavirus enhancer activator 3	X63190	4.55
EST	AA798971	2.32	Polypyrimidine tract binding protein	AV274525	2.55
EST	AI849939	2.28	Polypyrimidine tract binding protein 2	AI119718	2.3
EST	AI843155	2.27	Pore forming protein, perforin	X12760	2.56
EST	C79210	2.25	Praja1, similar to neurodegeneration associated protein 1	U06944	3.21
EST	AI847879	2.24	Praja1, similar to rat neurodegeneration associated protein 1	U06944	2.09
EST	AW121399	2.23	Protein kinase C, zeta	AV367375	2.4
EST	AW124529	2.23	Protein typrotein tyrosine kinase 9-like (A6-related protein)	Y17808	2.2

Table 1b. ***IL-12-inhibited genes (169 genes).***

Gene and description	Genbank accession	Fold inhibition	Gene and description	Genbank accession	Fold inhibition
EST	AV171460	2.22	RAB23, member RAS oncogene family	Z22821	2.25
EST	AA718040	2.21	rab6	L40934	2.04
EST	AW050015	2.2	Rbm14	X52102	2.09
EST	AW060927	2.19	Rearranged T-cell receptor beta V14/D1.1/J2.3 gene segment	X03278	2.41
EST	AW125116	2.18	RFC1	L23755	2.05
EST	AW046470	2.18	Rpl10	AV105022	2.24
EST	AW123921	2.17	s11-6, zinc finger protein	AB020542	2.14
EST	AW061306	2.17	Sepiapterin reductase	AI530375	2.24
EST	AV369210	2.17	Sex comb on midleg-like 1 (Drosophila)	AI853225	2.8
EST	AI845814	2.16	Sgne1, neuroendocrine protein 7B2	X15830	2.19
EST	AA867778	2.16	SHP2 interacting transmembrane adaptor	AJ236881	2.87
EST	AF110520	2.15	Solute carrier family 16 (monocarboxylic acid transporters), member 1	AF058055	2.04
EST	AA168476	2.14	Srm, spermidine synthase pseudogene 2	Z80833	2.37
EST	AW208513	2.13	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, C	Y09974	2.12
EST	AW049326	2.13	Tcra-V8	X06307	2.12
EST	AV084635	2.13	Tcrz, T cell receptor zeta chain	J04967	2.07
EST	AV314618	2.13	Thromboxane A2 receptor	D10849	2.44
EST	AI527477	2.11	Thy1	M12379	2.08
EST	AW060526	2.1	Thymoma viral proto-oncogene 2 (Akt2)	U22445	2.42
EST	AI007117	2.1	Tob, Trob	D78382	2.02
EST	AI854358	2.09	Translocase of inner mitochondrial membrane 22 homolog (yeast)	AA760359	2.08
EST	AI854144	2.07	TRAP, acid phosphatase type 5 gene	M99054	2.3
EST	AI846994	2.07	Ubiquitin-conjugating enzyme E2D 2	AV171056	2.33
EST	AA733372	2.05	Ubiquitin-conjugating enzyme E2H	U19854	2.21
EST	AA250414	2.05	UDP-N-acetyl-alpha-D-galactosamine	U18975	2.3
EST	AI850991	2.04	Upregulated by 1,25-dihydroxyvitamin D-3	AI839138	2.1
EST	AW123267	2.02	Zfp-35, zinc finger proetin	M36146	3.53
EST	AI596360	2.01	Zinc finger protein 161	AI447619	2.11
EST	AA688761	2.01	Zinc finger protein 54	AF080070	2.24

Table 1b. ***IL-12-inhibited genes (169 genes).***

Gene and description	Genbank accession	Fold inhibition	Gene and description	Genbank accession	Fold inhibition
EST	AV28333	2.01	Zinc finger protein 94	U46187	2.92