Characterization of Mouse and Human B7-H3 Genes

Mingyi Sun, Sabrina Richards, Durbaka V. R. Prasad, Xoi Muoi Mai, Alexander Rudensky and Chen Dong

*J Immunol* 2002; 168:6294-6297; doi: 10.4049/jimmunol.168.12.6294

http://www.jimmunol.org/content/168/12/6294

**References**

This article cites 17 articles, 3 of which you can access for free at:
http://www.jimmunol.org/content/168/12/6294.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
Characterization of Mouse and Human B7-H3 Genes

Mingyi Sun,* Sabrina Richards,† Durbaka V. R. Prasad,* Xoi Muoi Mai,* Alexander Rudensky,* and Chen Dong‡

T cell activation and immune function are regulated by costimulatory molecules of the B7 superfamily. Human B7-H3 is a recent addition to this family and has been shown to mediate T cell proliferation and IFN-γ production. In this work we describe the identification of the mouse B7-H3 homolog, which is ubiquitously expressed in a variety of tissues. Activated CD4 and CD8 T cells express a putative receptor that can be recognized by soluble mouse B7-H3-Ig molecules. While the mouse B7-H3 gene was found to contain a single copy, we discovered a novel isoform of human B7-H3 (named as B7-H3b hereafter) with four Ig-like domains that results from gene duplication and differential splicing. B7-H3b is the major isoform expressed in several tissues. This structural information suggests a genetic variation of the B7-H3 gene in mammalian species.

Materials and Methods

EST clones
Mouse B7-H3 and human B7-H3b expressed sequence tag (EST) clones (GenBank accession nos. BF450618 and BF984597, respectively) were purchased from Incyte Genomics (Palo Alto, CA). The plasmid DNA was prepared and sequenced by use of Big-Dye reagents from Qiagen (Valencia, CA).

Northern blot analysis
Mouse tissue Northern blot was purchased from Seegene (Seoul, Korea). A PCR fragment consisting of two Ig-like domains of the mouse B7-H3 gene was used for the analysis. The final wash of the blot was performed with 0.1× SSC/0.1% SDS at 65°C.

Expression and purification of B7-H3-Ig fusion protein
cDNA sequence encoding the extracellular portion of mouse B7-H3 protein was amplified by PCR and sequenced before subcloning into DES-Ig vector consisting of an insect expression plasmid pMT/BiP/V5-His A (Invitrogen, San Diego, CA) backbone and a human IgG1 Fc tag (a kind gift of Dr. E. Clark, University of Washington, Seattle, WA) (see Fig. 3A). Transfection of the new vector, which we named as DES-H3Ig, into the Drosophila cell line S2 results in CuSO4-inducible expression of B7-H3-Ig fusion protein in the culture supernatant.

Received for publication February 5, 2002. Accepted for publication April 8, 2002.

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

This study was supported in part by a start-up fund from the Department of Immunology at the University of Washington (Seattle, WA) and National Institutes of Health grant AI30746 to C.D. C.D. is the recipient of an Arthritis Investigator award from the Arthritis Foundation and a Basil O’Connor Starter Scholar Research award from the March of Dimes Foundation. A.R. is an Associate Investigator of the Howard Hughes Medical Institute.

M.S., S.R., and D.V.R.P. contributed equally to this work.

Address correspondence and reprint requests to Dr. Chen Dong, Department of Immunology, University of Washington, Box 357650, H466 Health Science Center, 1959 Northeast Pacific Street, Seattle, WA 98115-7650. E-mail address: chendong@u.washington.edu

Abbreviations used in this paper: ICOS, inducible costimulator; EST, expressed sequence tag; BLAST, basic local alignment search tool.


Copyright © 2002 by The American Association of Immunologists, Inc.

0022-1767/02/$02.00

Downloaded from http://www.jimmunol.org/ by guest on September 25, 2017
Analysis of human B7-H3 gene splicing

Human B7-H3b cDNA, which consists of two pairs of IgV-IgC domains, was PCR-amplified from a human colon cDNA and sequenced. The primers used are the forward, agcactgtggttctgcctca, and the reverse, tgcattctcctcctcacagc. To analyze B7-H3 splicing in different tissues, a pair of primers designed from the common sequences of B7-H3a and B7-H3b, which lie in exons 1 and 8 of the B7-H3 gene (see Fig. 4A), was used to amplify cDNA samples of multiple human tissue cDNA panel (Clontech Laboratories, Palo Alto, CA).

Sequence analysis

The Ig domains of mouse B7-H3 protein were predicted by CD-Search program of the National Center for Biotechnology Information. Genomic structure of mouse and human B7-H3 genes was analyzed by use of standard nucleotide basic local alignment research tool (BLASTN) and translate BLAST (TBLASTN) methods in the public human genome database and in the mouse and human genome database of Celera (Rockville, MD). The alignment of mouse and human B7-H3 protein sequences was performed using Jellyfish software (Biowire, San Francisco, CA). Comparison of mouse B7-H3 and other B7 family members was performed using basic local alignment search tool 2 of the National Center for Biotechnology Information, and the phylogeny tree was constructed with GeneWorks software (Gene Works, Australia).

Results and Discussion

T cell activation and function are regulated by costimulatory molecules, most notably those of the B7 family (1). It has been recently estimated that ~20 B7 family members exist in humans.
(17). Therefore, structural and functional analysis of B7 family genes will benefit our understanding of the regulation of the adaptive immune system.

Human B7-H3 is among the most recently discovered B7-like molecules (16). Soluble human B7-H3 molecule was previously reported to bind to activated T cells and costimulate their proliferation and IFN-γ production (16). To characterize the mouse counterpart, we did a search of the mouse EST database and discovered a clone with significant homology with the described human sequence. We obtained the clone and completely sequenced the coding region. The predicted mouse B7-H3 protein contains 315 amino acids (Fig. 1A) with 88% identity and 93% similarity with the human molecule, while sharing ~20–30% identity with the other B7 family members known to date (Fig. 1B). Murine B7-H3 has a leader peptide and a transmembrane domain, indicating that, as the human molecule, it is a type 1 transmembrane glycoprotein. The extracellular portion of mouse B7-H3, like those in many other B7 family members, consists of one IgV- and one IgC-like domain (Fig. 1A).

We then examined the expression of mouse B7-H3 mRNA in multiple tissues by Northern blot analysis. Quite similar to its human homolog (16), mouse B7-H3 is widely expressed in all tissues we analyzed (Fig. 2). The size of its mRNA is ~1 kb bigger than that of the EST clone, suggesting that the transcript has a long 3′ untranslated region. Ubiquitous expression of B7-H3 in nonlymphoid tissues suggests its role in modulating immune responses in these tissues. However, it is unclear at this stage what specific cell types express B7-H3.

To assess whether mouse B7-H3, like its human counterpart, binds to a receptor on activated T cells, we produced soluble mouse B7-H3 protein with a human IgG1 Fc tag (Fig. 3B). When we used biotinylated fusion protein to examine expression of a putative mouse B7-H3 receptor, we found that the soluble protein does not bind significantly to CD4 or CD8 cells from C57BL/6 lymph node cells (Fig. 3C). After these cells were activated with 2.5 μg/ml Con A for 48 h, they could all be recognized by the fusion protein (Fig. 3C). This result suggests a mouse B7-H3 receptor is induced upon T cell activation. Therefore, the B7-H3
ligand-receptor interaction may possess a potential function in modulating T cell effector activity.

When we searched the human EST database with mouse B7-H3 amino acid sequences, we found several of them with minor sequence differences with the reported B7-H3 sequence. We ordered one of the clones and completely sequenced it. This clone appears to encode a novel isoform of human B7-H3, because it has a different N-terminal sequence and a few amino acid substitutions in the two extracellular Ig domains (Fig. 4A). However, the rest of the coding sequence is identical to that of B7-H3. We name this new isoform B7-H3b. The sequence we obtained from our own analysis was confirmed by those of multiple clones in the EST database, indicating that the difference we observed between B7-H3 and B7-H3b was not an artifact. The alignment of human B7-H3, B7-H3b, and mouse B7-H3 is shown in Fig. 4A. Although the human B7-H3 and mouse B7-H3 have similar N-terminal amino acid sequences, the extracellular portion of B7-H3b and mouse B7-H3 share closer homology.

To identify the molecular origin of B7-H3 and B7-H3b isoforms, we searched the public human genome database using B7-H3 and B7-H3b nucleotide and amino acid sequences. A fragment of human chromosome 15 contains sequences homologous to both isoforms (Fig. 4B). From this analysis, we deduced the genomic structure of the human B7-H3 locus (Fig. 4B), which was confirmed by analysis using the Celera database (data not shown). It appears the B7-H3 and B7-H3b isoforms are the result of a gene duplication of two exons encoding the IgV-IgC domains of the B7-H3 molecule. These two isoforms share the same exons 4–7, which result in the identical transmembrane and intracellular sequences as described above (Fig. 4A).

Interestingly, the most N-terminal amino acids present in the B7-H3b EST clone we sequenced lie in the middle of exon 3 of the B7-H3 gene (Fig. 4B). Because there is no stop codon in front of the first ATG sequence, this raised a possibility that the EST may not reflect the full-length B7-H3b-encoding cDNA, which may be even longer at the 5’ and may contain two pairs of IgV-IgC domains. To assess this, we designed a pair of primers corresponding to the sequence from exons 1 and 8 (Fig. 4B), respectively, so that they would amplify a 1300-bp product if there were an isoform containing four Ig domains and 600 bp for the two-Ig isoform. When we used a template human colon cDNA, which gave high B7-H3 expression in the Northern blot analysis (16), we observed a 1300-bp PCR product (data not shown). Subsequent DNA sequencing analysis confirmed that this product does correspond to the four-Ig isoform (data not shown). In this PCR analysis, a 600-bp product was also found, although present at much lower levels (data not shown).

To assess whether there is any tissue-specific splicing of the B7-H3 gene, we performed analogous PCR analysis of a cDNA panel of multiple human tissues (Clontech Laboratories) (Fig. 4C). In most tissues examined, except for brain and placenta, B7-H3b represented the major isoform. Interestingly, placenta does not express B7-H3b at all. When we sequenced the 600-bp PCR products from several tissues, we found that they contain the first IgV-IgC domains (data not shown). Therefore, they are the previously described B7-H3b isoform.

To assess whether the mouse B7-H3 gene has multiple isoforms, we searched the Celera mouse genome database and found seven exons encoding the B7-H3 protein on chromosome 9. There is no evidence of gene duplication in this case. This indicates that the two B7-H3 isoforms in humans must be derived by a duplication of two exons after separation of human and mouse ancestors. The functional significance of this gene duplication event is unclear right now, although it is possible that B7-H3b molecules with four Ig-like domains may ligate two receptors at once and therefore be a stronger cross-linking ligand. Nonetheless, in light of the potentially important immunomodulatory function of B7 family member, it is interesting that the mammalian species are still evolving new molecules to regulate the adaptive immune function.

In summary, we report in this work, for the first time, the identification and expression pattern of human homolog of B7-H3, and a new human B7-H3b isoform generated by gene duplication and differential splicing. Our results revealed a variation of this gene in mammalian species. B7-H3 molecules are widely expressed in mouse and humans and therefore may possess fundamental immunoregulatory roles, especially in nonlymphoid tissues, to engage receptors on activated T cells. With the structural information described in this paper and the reagents prepared from it, exciting data may emerge in the near future on the function and regulation of B7-H3 as a novel costimulatory molecule.

Acknowledgments

We thank Dr. Edward Clark for human Ig plasmid, Caroline Bishop for the phylogenetic analysis of B7 family proteins and critical reading of the manuscript, and the entire Dong Laboratory for their help and discussion.

References