Small Molecule Ligands Define a Binding Site on the Immune Regulatory Protein B7.1*

David V. Erbe‡, Suyue Wang, Yuzhe Xing, and James F. Tobin

From Wyeth Research, Cambridge, Massachusetts 02140

The interaction of co-stimulatory molecules on T cells with B7 molecules on antigen presenting cells plays an important role in the activation of naive T cells. Consequently, agents that disrupt these interactions should have applications in treatment of transplant rejection as well as autoimmune diseases. To this end, specific small molecule inhibitors of human B7.1 were identified and characterized. These compounds inhibit the binding of B7.1 to both CD28 and CTLA4. Both classes of compounds appear to bind the same site, a relatively small portion of the GFCC" face of the N-terminal V-set domain of human B7.1, not present in the homologous B7.2 or even mouse B7.1. This site may represent a rare hot spot for small molecule antagonist design of inhibitors of cell-cell interactions, whose ligands may yield leads for the development of novel immunomodulatory medicines.

Full T cell activation requires both an antigen-specific and a second co-stimulatory signal. Co-stimulation dictates the outcome for T cells through the binding of B7.1 (CD80) and B7.2 (CD86) expressed on antigen-presenting cells to CD28 and CTLA4 on T cells (1, 2). Signaling through CD28 augments the T cell response, whereas CTLA4 signaling attenuates it (3–5). Animal studies and clinical trials with protein antagonists of these interactions indicate considerable promise for immunotherapy in transplantation and autoimmune disease (1, 2, 6–10).

Identification of potent, orally active, small molecule inhibitors of the extracellular binding of these co-stimulatory molecules faces a number of thermodynamic challenges common to protein-protein interactions between opposing cell surfaces. Principally, these present obstacles to identifying inhibitors with sufficient binding kinetics (slow enough off-rates) to compete effectively. Protein-protein interfaces can be large and relatively shallow (11, 12), without preferred sites for small ligands. Additionally, the multiple interactions between the two cell surfaces results in a high avidity. Further still, T cell signaling occurs in a specialized contact area between cells, termed the immunological synapse (13, 14) that forms to concentrate receptors and their signaling complexes (15). Although some successes have been reported for small molecule antagonism of analogous interactions mediated by integrins (16–19) and selectins (20, 21), co-stimulatory molecule interactions may present a difficult target. This is especially true for interactions involving CTLA4, whose submicromolar affinity for B7.1 is unusually high for interactions between cell surface molecules (22). In the recently solved crystal structure of the human CTLA4/B7.1 complex, CTLA4 and B7.1 pack in a strikingly periodic zipper-like arrangement in which bivalent CTLA4 homodimers bridge bivalent B7.1 homodimers. This reveals the structural basis for formation of unusually stable signaling complexes (23). The combination of avidity-driven binding and submicromolar affinity is thought to be unique to CTLA4 and B7.1 (27). Although CD28 binds B7 molecules with a lower affinity than CTLA4 (22), each of the other challenges mentioned above for inhibiting protein-protein interactions applies to small molecule antagonism of CD28 binding to the B7s as well.

Consequently, the small molecule binding site on human B7.1 identified here may represent an exceptional, albeit challenging opportunity. In a high throughput screen we identified a number of inhibitors of B7.1/CD28 binding, two classes of which were studied further. Equilibrium dialysis demonstrated that these compounds bound specifically to human B7.1 at a common site. Occupancy of this site by the inhibitors blocked B7.1 binding not only to CD28, but also to CTLA4, although at much higher concentrations of inhibitors. Mapping of the binding of these small molecules then located this unique site to the GFCC" face of the N-terminal V-set domain of human B7.1, very near to the site of its interactions with counter receptors.

**EXPERIMENTAL PROCEDURES**

Reagents—Production and characterization of antibodies to human B7.1 (EW3.1F1 and EW3.3B5), B7.1-Fc chimeras (including domain swaps and point mutants), and CTLA4-Fc were as described previously (23). CD28-Fc was produced similarly and kindly provided by Drs. James Wilhelm and Pranab Chanda. Fab fragments of the 1F1 antibody were made by digestion with papain and purified by protein-A affinity chromatography. The affinity of the Fab fragments for human B7.1 were determined with a BIACore 2000 by surface plasmon resonance measurements using immobilized human B7.1. A calculated Kd was obtained by first determining the dissociation rate constant, koff, and then the association rate constant, kao, was determined by fitting the data contained in the dissociation phase of the sensorgram to a monoeponential decay function. koff was determined by fitting the data contained in the associations phase of the sensorgram to the integrated rate equation using the calculated kao.

CD28/B7.1 ELISA—ELISA wells were coated with 300 ng of CD28-Fc in carbonate buffer (pH 9.4) overnight at 4 °C, blocked with 1% bovine serum albumin in TBS for 1 h at 22 °C and then washed three times in TBS prior to assay. The detection complex was formed as follows: B7.1-Fc-biotin, prepared using NHS-LC-biotin (Pierce 21335) according to the manufacturer’s instructions (4.1 mol of biotin/mol of Fc) was added at 0.8 μg/ml to streptavidin-alkaline phosphatase (Caltag SA1008) at 1:1000 in TBS. Inhibitors or Me2SO (% final) were added to this complex and incubated 30 min at 22 °C. Detection complex

Received for publication, October 22, 2001, and in revised form, December 3, 2001
Published, JBC Papers in Press, December 6, 2001, DOI 10.1074/jbc.M110162200

* 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.

‡ To whom correspondence should be addressed. Tel.: 617-665-5430; Fax: 617-665-5390; E-mail: derbe@genetics.com.

‡ The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; TBS, Tris-buffered saline; Me2SO, dimethyl sulfoxide; pNPP, p-nitrophenyl phosphate; CHO, Chinese hamster ovary.

This paper is available online at http://www.jbc.org

7363
(± inhibitors) was then added to the CD28-coated wells for 25 min at 22 °C, washed five times with TBS, developed with the colorimetric substrate pNPP (Pierce 34045) in diethanolamine/MgCl₂ buffer (pH 9.5) and read at 405 nm. The high-throughput screen for antagonists used this CD28/B7.1 ELISA in an automated format to test the Wyeth Research library of proprietary compounds, a collection resulting from both historical in-house synthetic efforts as well as compounds acquired from commercial and academic sources.

**CTLA4/B7.1 ELISA—**Wells were coated with 300 ng of CTLA4-Fc in phosphate-buffered saline overnight at 4 °C, blocked with 1% bovine serum albumin in TBS for 1 h at 22 °C, and then washed three times in TBS prior to assay. B7.1-Fc-biotin was added at 0.5 µg/ml with or without inhibitors for various time points before washing five times in TBS. Streptavidin-alkaline phosphatase at 1:1000 in TBS was then added for 1 h for detection, followed by washing and color development with pNPP as above. For the antibody washout experiments, after washing out unbound B7.1-Fc-biotin, anti-B7.1-blocking antibody 1F1 was added at 10 µg/ml for various times prior to re-wash and detection with streptavidin-alkaline phosphatase.

**Equilibrium Dialysis—**Dialysis experiments were performed in teflon microdialysis chambers from Amicon by dissolving compounds and proteins in phosphate-buffered saline and placing each on opposite sides of a 5,000 molecular weight cutoff, regenerated cellulose membrane, and allowing to stand 1–3 days. To quantify compound concentrations, an aliquot was removed from each chamber side, an equal volume of acetonitrile/0.15% trifluoroacetic acid was added, followed by vortexing and spinning at 12,000 g to pellet precipitated protein. Supernatant samples were loaded onto a monomeric C₁₈ column from Vydac (cat. 238TP54) equilibrated with water/0.15% trifluoroacetic acid at a flow rate of 0.6 ml/min and eluted with a 30-min gradient from 0 to 90% acetonitrile/0.15% trifluoroacetic acid. Absorbance was monitored at various wavelengths and compared versus standards for quantification of the integrated peaks. Unless otherwise indicated, proteins were used at 10 µM with compounds at 5 µM prior to any binding equilibria. All solutions contained 1% Me₂SO. For antibody blocking experiments, each monoclonal was added at a molar excess to each side of the dialysis membrane. For the small molecule competition experiments, dialysis chambers with B7.1-Fc and compound 1 were equilibrated as above along with a 14-fold molar excess of compound 2. Aliquots from each chamber were then extracted in CH₃Cl to separate compound 1 from compound 2 (which remains in the aqueous phase). The CH₃Cl was then evaporated, and the compound 1 was dissolved in Me₂SO and loaded onto the C₁₈ column for quantification as before.

**Cell Adhesion—**Stable CHO cell lines were established expressing full-length human B7.1 or CD28. CHO/CD28 cells were grown to confluence and washed with phosphate-buffered saline prior to assay. CHO/B7.1 cells were harvested by incubating with EDTA, scraping and washing in assay buffer (Hanks’-buffered saline solution + 2% fetal calf serum). CHO/B7.1 cells were then labeled with 5 µM Calcein AM (Molecular Probes, cat. C-3099) for 20 min at 37 °C, washed in assay buffer, and re-suspended at 10⁶ cells/ml. Labeled CHO/B7.1 cells were incubated with inhibitors for 30 min at 37 °C, added to plates of confluent CHO/CD28 cells, and incubated 30 min at room temperature with occasional shaking. Plates were then gently washed with assay buffer by pipetting and aspirating. Bound fluorescent cells were quantified by exciting at 485 nm and measuring the resulting emission at 530 nm.

![Fig. 1. Structures of human B7.1 inhibitors.](image)

**RESULTS**

In a screen for antagonists of the binding of CD28 to B7.1, a number of compounds were identified, which reversibly inhibited this interaction with IC₅₀ values in the nanomolar range. Two of these, compound 1 (IC₅₀ = 60 ± 17 nM) and compound 2 (IC₅₀ = 30 ± 6 nM) whose structures are shown in Fig. 1, were selected for further characterization. Equilibrium dialysis employing various receptor-Fc chimeras was used to establish the binding partner and specificity for each compound. As shown in Fig. 2, compound 1 is selectively enriched only in the presence of the human B7.1-Fc protein. No binding to CD28-Fc, CTLA4-Fc, B7.2-Fc or a control IgG1 Fc was detected. Importantly, this compound also did not bind to mouse B7.1-Fc. An identical binding pattern with these proteins was seen for the second compound, compound 2 (not shown). Thus, these two compounds appear to bind specifically to human B7.1 and not to the very related mouse B7.1 homolog. These results were confirmed by inhibition experiments in which neither compound inhibited CD28’s interaction with B7.2 or mouse B7.1 when tested by ELISA at concentrations as high as 10 µM (not shown).

Because these small molecules do not bind the mouse B7.1 protein, their binding epitopes can be mapped based upon amino acid differences between the two species, as was recently reported for a small molecule that binds to the glucagon-like peptide 1 receptor (GLP-1 R) (25). Additionally, one can use the known binding epitopes of antibodies to human B7.1 to explore small molecule binding as was done for an LFA-1 antagonist (17). We had previously constructed human-mouse mutant B7.1 chimeras in which mouse residues were substituted for their human counterparts in the human B7.1-Fc chimera background.¹ These were then used to map the binding epitopes for an entire panel of blocking antibodies to the GFCC C⁺ face of the N-terminal V-set domain of human B7.1.¹ Fig. 3 shows the results when these reagents are employed in equilibrium dialysis experiments with each of the small molecules. Compound 1 appears to bind to the N-terminal V-set domain of human B7.1. One sees binding with a construct containing the human V-set domain fused to the mouse C1-set domain (hVmC1) and not with a construct containing the mouse V-set domain fused to the human C1-set domain (mVhC1) (Fig. 3A). Furthermore, preincubation of human B7.1-Fc in the presence of an antibody specific for the V-set domain prevented binding of compound 1, whereas preincubation with a C1-set domain-specific antibody had no effect (Fig. 3A). As seen in Fig. 3B, compound 2 also appears to bind the V-set domain, one again sees binding with hVmC1 and not with mVhC1. The human B7.1 V-set domain appears to be sufficient for binding of compound 2 because a
construct consisting of only this domain fused directly to the IgG1 Fc gives enrichment in the dialysis chamber (Fig. 3B).

In fact, these two small molecules appear to share a binding site within this N-terminal domain of B7.1. A 14-fold molar excess of compound 2 is able to significantly block binding of compound 1 to B7.1 (Fig. 4). This common binding site is shared (at least in part) with the blocking antibody to B7.1 (EW3.1F1), which binds to residues within the two loops I41-I49 and L85-E95 on the GFC/C' face of the B7.1 N-terminal IgSF domain as previously reported. Consequently, human B7.1-Fc chimeras with amino acid substitutions in and near these loops were used in dialysis experiments (Fig. 5). Here, we see similar (although not identical) binding patterns for the two small molecules. Compound 1 does not bind a B7.1 chimera with the loop D46-I49 mutated (Fig. 5, M2) nearly as well as wild type B7.1. It also does not bind a construct with the neighboring residue (Trp-50) mutated to alanine (Fig. 5, M3). Compound 2 also shows diminished binding to these two mutants, although not as much. Control mutants away from this region (M1, M4) showed no differences with either small molecule. Importantly, mutation of Trp-50 to Ala in B7.1 also results in loss of binding to both of B7.1’s natural ligands (26), indicating a possible direct overlap in the binding epitopes for both small molecules identified here with CD28 and CTLA4. Because the binding sites for these small molecules appeared to overlap with the proposed sites for both CD28 and CTLA4, their ability to block B7.1’s binding to CD28-Fc was tested. When tested near their IC_{50} values for inhibition of CD28 binding, no inhibition of CTLA4 binding to B7.1 in the ELISA by either compound was seen (not shown). This is possibly due to the fact that the dissociation rate constant of CTLA-4 for B7.1 is slower than that of CD28 for B7.1, and could even reflect a difference in stoichiometry if CD28 binds monovalently to B7.1 as opposed to the multivalent binding, which has been demonstrated for CTLA4 (22, 23, 27). Therefore, more extensive analysis of small molecule inhibition of B7.1 binding to CTLA4 was pursued at higher concentrations and various time points (Fig. 6). Here, the CTLA4/B7.1 ELISA was used to measure inhibition by compound 1 at various times, and at a concentration of 10 μM, more than 100 times its IC_{50}, for inhibition of CD28 binding to B7.1. One sees clear inhibition at this concentration during early time points (~65%), which then decreases as the binding is allowed to continue beyond 1 h. Thus, over time, CTLA4 appears to compete more effectively for B7.1 than the small molecule. Control experiments with CD28-Fc showed no such decrease in inhibition over time (not shown). Furthermore, even after 4 h of CTLA4/B7.1 binding in the ELISA, addition of a blocking antibody to B7.1, with a much slower dissociation rate constant, is able to effectively compete with CTLA4 (Fig. 6). Thus, B7.1/CTLA4 binding at these later time points is still at an equilibrium, which the antibody inhibits while the small molecule does not. These data confirm that the small molecule binding site on B7.1 identified here is likely near both the binding sites for CD28 and CTLA4.

Blockade of co-stimulatory signals through CD28 and CTLA4 would be expected to have the opposite effect in disease states. So, this difference in potency in inhibition by these small molecule antagonists of B7.1 could provide an advantage if a concentration of inhibitor could be used which would distinguish between CD28 and CTLA4 inhibition. Therefore, we next explored the concentrations of compound 1 needed to inhibit CD28/B7.1 interactions in cell-based assays. In simple
adhesion assays using COS cells overexpressing CD28 adhering to cells overexpressing B7.1, no inhibition by compound 1 was seen at concentrations up to 100 nM (Table I). Furthermore, in a variety of assays dependent on B7.1-mediated T cell co-stimulation through CD28, no specific inhibition was seen with compound 1 at up to 10 nM (not shown). As with inhibition of CTLA4 binding by B7.1, this was likely due to the relatively fast off-rate of the small molecule and its inability to compete with the avid interactions between cells. To explore this, we generated a monovalent Fab fragment of the blocking antibody EW3.1F1. This Fab, with a 10 nM $K_d$ for B7.1 binding by surface plasmon resonance and a dissociation rate constant 20 times faster than the divalent parent antibody (Fig. 7A), inhibited CD28/B7.1 binding with an IC$_{50}$ in this range (3.4 ± 0.3 nM, Table I). We then selected an analog of compound 1, compound 3, which was more than 10-fold more potent in the CD28 ELISA (Table I) with an IC$_{50}$ of 4 nM. This molecule, whose structure is shown in Fig. 7B, resulted from a detailed synthesis effort directed at improved potency for CD28/B7.1 inhibition which we have reported separately. Importantly, it has an IC$_{50}$ in the B7.1/CD28 ELISA equivalent to the antibody Fab fragment. When these two reagents are used in a cell-based assay of B7.1/CD28 binding, however, one sees different results. No inhibition by compound 3 is seen at concentrations up to 100 nM, while the Fab fragment inhibits with an IC$_{50}$ of 900 nM. This shift in potency for the Fab fragment from 3 nM in the ELISA to 900 nM in cells probably reflects the effects of increased avidity present in the cell-based assay. Although the Fab fragment and compound 3 may have similar binding constants for B7.1 (as estimated from their ELISA IC$_{50}$), the small molecule probably has much faster on- and off-rates. This faster off-rate probably limits its ability to inhibit CD28 interactions in the cellular milieu. (Our attempts to measure this dissociation rate constant of the small molecule for B7.1, indicated an off-rate faster than the limits of the assay (seconds).) Importantly, more sensitive tests may be required to see an effect of these inhibitors in cells, such as examining early signaling events upon stimulation of CD28 by B7.1.

**DISCUSSION**

Some of the most desirable drug targets may also be the most challenging to inhibit with small molecules: protein-protein interfaces consisting of relatively shallow surfaces without
binding sites for small ligands. Although thermodynamically favored hot spots are sometimes used to drive protein-protein binding (29), the occurrence of invaginations sufficient for high affinity small molecule binding (30) may be limited in the protein universe. In fact, although computational methods can identify numerous sites on protein surfaces for potential ligands, very few of these sites are ever seen experimentally, perhaps due to solvent effects (31-34). Consequently, recent efforts have sought to permit identification of even low affinity (micromolar to millimolar) ligands for proteins that can then be systematically optimized (and even combined) for improved affinity (24, 28). The results of such efforts emphasize the challenges to small molecule intervention at protein interfaces. Importantly, the results presented here indicate that a small molecule binding pocket does exist on B7.1, one which is sufficient for the binding of at least two classes of small molecules with nanomolar affinities. The evidence indicates that this pocket may be unique among the B7 family of co-stimulatory molecules.

This binding site for small molecules on B7.1 maps near its counter receptor binding site, which has been recently characterized by crystallography (23). The crystal structure of B7.1 alone revealed a protein with an amino-terminal domain with the immunoglobulin V-set topology followed by a membrane proximal domain with C1-set topology (27). In contrast to other interacting cell surface molecules, B7.1 binds CTLA4 with a relatively small interface with an unusually high degree of shape complementarity, which accounts for their binding kinetics (11, 22, 23). In the co-crystals with CTLA4, it is only the N-terminal V-set domain of B7.1 that contacts CTLA4 (23). Thus, it is likely that this domain of B7.1 also makes all contacts with CD28 for reasons previously outlined (23). Of particular importance are two surface loops on the GFCC/C face of this domain. B7.1/CTLA4 binding is dominated by the loop of CTLA-4 which contains the conserved, hydrophobic MYPPPYY sequence contacting a largely nonpolar surface of B7.1 consisting of Tyr-31, Met-38, Thr-41, Met-43, Val-83, Leu-85, Ala-91, Phe-92, and Leu-97 (23). These contacts probably contribute the majority of the binding energy. Specificity at the binding interface is likely added through hydrogen bonding including a dominant one between Glu-33 on CTLA-4 and Arg-29 on B7.1. This region of B7.1 is likely the sole determinant for CD28 and CTLA4 binding, as emphasized by the fact that binding epitopes for an entire panel of blocking antibodies also mapped here.

Although the small molecule antagonists appear to bind near this site, upon examination of the structure of B7.1 in this region, no obvious cavity is apparent (see Refs. 23 and 27). It
seems possible, therefore, that some surface loop movement creates a cleft or pocket that then permits small molecule binding to occur. Interestingly, binding of the inhibitors to B7.1 is time-dependent (data not shown) and could be explained by a loop movement within B7.1 or an energetic change in the small molecules themselves. It is also of interest to note that the two separate classes of antagonists identified here appear to bind to this same site. Thus, this portion of B7.1 may represent a fortuitous occurrence of a thermodynamically favored small molecule binding site, one which is not present in mouse B7.1 or in human B7.2. These results highlight the importance of this region along the GFC'C' face of the V-set domain as the crucial site for B7.1 antagonist design. That the small molecule B7.1 antagonists reported here do not inhibit CD28-mediated co-stimulation in cell-based readouts perhaps indicates the limits of such an approach at blocking high avidity protein-protein complexes. It is possible that through B7.1 co-structures with bound inhibitors, more potent compounds (with slower off-rates) can be synthesized. Such compounds could provide a novel means of inhibiting co-stimulation, yielding an exciting approach to immune modulation in autoimmune disease and graft acceptance in transplantation.

Acknowledgements—We thank Christina Yoon, Lydia Mosyak, Will Somers, and Mark Stahl for helpful advice and discussion, Neal Green, Jason Xiang, Gary Stack, and Arthur Santilli for providing compounds, Rich Zollner for conditioned media production, Yan Zhang for Fc chimera purification, Susan Bernard and Tony Li for DNA sequencing, Tom Novak for high throughput screening, and the Antibody Technology Group for production and purification of anti-B7.1 monoclonal antibodies.

REFERENCES
1. Greenfield, E. A., Nguyen, K. A., and Kuchroo, V. K. (1998) Crit. Rev. Immunol. 18, 389–418
2. Lenschow, D. J., Walunas, T. L., and Bluestone, J. A. (1996) Annu. Rev. Immunol. 14, 233–258
3. Harding, F. A., McArthur, J. G., Gross, J. A., Raulet, D. H., and Allison, J. P. (1992) Nature 356, 607–609
4. Walunas, T. L., Lenschow, D. J., Bakker, C. Y., Linsley, P. S., Freeman, G. J., Green, J. M., Thompson, C. B., and Bluestone, J. A. (1994) Immunity 1, 465–481
5. Waterhouse, P., Penninger, J. M., Timms, E., Wakeham, A., Shahinian, A., Lee, K. P., Thompson, C. B., Giresser, H., and Mak, T. W. (1995) Science 270, 985–988
6. Abrams, J. R., Kelley, S. L., Hayes, E., Kikuchi, T., Brown, M. J., Kang, S., Lebwohl, M. G., Guzzo, C. A., Jegasothy, B. V., Linsley, P. S., Krueger, J. G. (2000) J. Exp. Med. 192, 681–694
7. Guinan, E. C., Boussetiotis, V. A., Neuberger, D., Brennan, L. L., Hirano, N., Nadler, L. M., and Gribben, J. G. (1999) N. Engl. J. Med. 340, 1704–1714
8. Larsen, C. P., Elwood, E. T., Alexander, D. Z., Ritchie, S. C., Hendrix, R., Tucker-Burden, C., Cho, H. R., Aruffo, A., Hollenbaugh, D., and Linsley, P. S. (1996) Nature 381, 434–438
9. Leach, D. R., Krummel, M. F., and Allison, J. P. (1996) Science 271, 1734–1736
10. Linsley, P. S., Wallace, P. M., Johnson, J., Gibson, M. G., Greene, J. L., Ledbetter, J. A., Singh, C., and Tepper, M. A. (1992) Science 257, 792–795
11. Janin, J., and Chothia, C. (1990) J. Biol. Chem. 265, 16027–16030
12. Lawrence, M. C., and Colman, P. M. (1993) J. Mol. Biol. 234, 846–850
13. Grakoui, A., Bromley, S. K., Samee, C., Davis, M. M., Shaw, A. S., Allen, P. M., and Dustin, M. L. (1999) Science 285, 221–227
14. van der Merwe, P. A., Davis, S. J., Shaw, A. S., and Dustin, M. L. (2000) Semin. Immunol. 12, 5–21
15. Lee, K.-M., Chuang, E., Griffin, M., Khattri, R., hong, D. K., Zhang, W., Straus, D., Samelson, L. E., Thompson, C. B., and Bluestone, J. A. (1998) Science 282, 2263–2266
16. Chen, L. L., Whitty, A., Leb, R. R., Adams, S. P., and Pepinsky, R. B. (1999) J. Biol. Chem. 274, 13167–13175
17. Kelly, T. A., Jeanfavre, D. D., McNeil, D. W., Adames, S. P., Reilly, P. L., Mainolfi, E. A., Kishimoto, K. M., Nabony, G. H., Zinter, R., Bornman, B.-J., and Rothlein, R. (1999) J. Immunol. 163, 5173–5177
18. Lin, K., Ateehq, H. S., Hsiung, S. H., Chong, L. T., Zimmermann, C. N., Castro, A., Lee, W., Hammond, C. E., Kalkunte, S., Chen, L.-I., Pepinsky, R. B., Leone, D. B., Sprague, A. G., Abraham, W. M., Fill, A., Leb, R. R., and Adams, S. P. (1999) J. Med. Chem. 42, 920–934
19. Miller, W. H., Keenan, R. M., Willette, R. N., and Lark, M. W. (2000) Drug Disc. Today 5, 397–408
20. Kaila, N., Xu, Y., Camphaussen, R. T., and Xiang, Y. (2001) Bioorg. Med. Chem. 9, 801–806
21. Skee, D. H., Romano, S. J., Yu, J., Nguyen, T. N., John, J. K., Raheja, N. K., Axe, P. U., Jones, T. K., and Ripka, W. C. (2001) J. Med. Chem. 44, 2094–2107
22. van der Merwe, P. A., Bodian, D. L., Daenen, S., Linsley, P., and Davis, S. J. (1997) J. Exp. Med. 185, 393–403
23. Stamper, C. C., Zhang, Y., Tobin, J. F., Erbe, D. V., Ikenmizu, S., Davis, S. J., Stahl, M. L., Seehra, J., Somers, W. S., and Mosyak, L. (2001) Nature 410, 608–611
24. Erlanson, D. A., Braisted, A. C., Raphael, D. R., Mandal, M., Stroud, R. M., Gordon, E. M., and Wells, J. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9367–9372
25. Tibauduia, E. C., Chen, C., and Beinborn, M. (2001) J. Biol. Chem. 276, 37787–37793
26. Fargeas, C., Trunke, A., Reddy, M., Hurle, M., Sweet, R., and Sekaly, R. P. (1995) J. Exp. Med. 182, 667–675
27. Ikenmizu, S., Gilbert, R. J. C., Fennelly, J. A., Collins, A. V., Harlos, K., Jones, E. Y., Stuart, D. I., and Davis, S. J. (1997) J. Exp. Med. 182, 9367–9368
28. Shuker, S. B., Hajduk, P. J., Meadows, R. P., and Pies, S. W. (1996) Science 274, 1531–1534
29. Clackson, T., and Wells, J. A. (1995) Science 267, 383–386
30. Kunz, I. D., Chen, K., Sharp, K. A., and Kollman, P. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10007–10002
31. Ringe, D., and Mattos, C. (1999) Med. Res. Rev. 19, 321–331
32. Miranker, A., and Karpplus, M. (1995) Protein 11, 29–34
33. Goodford, P. J. (1985) J. Med. Chem. 28, 849–857
34. Allen, K. N., Bellamacina, C. R., Ding, X. C., Jeffery, C. J., Mattos, C., Petsko, G. A., and Ringe, D. (1996) J. Phys. Chem. 100, 2665–2671
Small Molecule Ligands Define a Binding Site on the Immune Regulatory Protein B7.1
David V. Erbe, Suyue Wang, Yuzhe Xing and James F. Tobin

J. Biol. Chem. 2002, 277:7363-7368.
doi: 10.1074/jbc.M110162200 originally published online December 6, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M110162200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 16 of which can be accessed free at
http://www.jbc.org/content/277/9/7363.full.html#ref-list-1