**DAB$_{389}$ Interleukin-2 Receptor Binding Domain Mutations**

CYTOTOXIC PROBES FOR STUDIES OF LIGAND-RECEPTOR INTERACTIONS*

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Site-directed mutagenesis was used to generate point mutations in the diphtheria toxin-related fusion protein, DAB$_{389}$ interleukin-2 (IL-2), Thr-439, in the IL-2 receptor binding domain of the fusion toxin, was changed to a Pro residue. The resultant fusion toxin, DAB$_{389}$ IL-2(T439P), was 300-fold less cytotoxic than wild type DAB$_{389}$ IL-2, partially as the result of a 100-fold decrease in binding affinity for the high affinity form of the IL-2 receptor. However, DAB$_{389}$ IL-2(T439P) stimulated DNA synthesis to a greater extent than expected. Studies of intoxication kinetics indicated that the increased stimulation might result from an increased contact time between the mutated IL-2 receptor binding domain and the receptor, perhaps due to a decreased internalization rate. Another mutant, DAB$_{389}$ IL-2(Q514D), in which a Gln residue at position 514 was changed to an Asp, was 2000-fold less cytotoxic than wild type DAB$_{389}$ IL-2. This mutant had a 50-fold decrease in binding affinity, did not stimulate DNA synthesis and also had a reduced rate of intoxication. Gln-514 appears to play a role in receptor binding and activation, whereas Thr-439 appears to be involved with receptor binding and signaling internalization of the fusion toxin receptor complex.

Interleukin-2 (IL-2)$^1$ is a 133-amino acid lymphokine protein secreted by activated T-cells. Binding of IL-2 to its high affinity receptor stimulates processes that result in gene activation, DNA synthesis, internalization of the IL-2-IL-2 receptor complex, and proliferation of IL-2-dependent T cells (1–6). The high affinity form of the IL-2 receptor is composed of at least three subunits, $\alpha$, $\beta$, and $\gamma$. The $\alpha$ subunit forms a heteromeric complex with the $\beta$ subunit that functions to bind IL-2 to the surface of activated T cells (7). The $\beta$ and $\gamma$ subunits associate in a ligand-dependent fashion and appear to be involved with intracellular signaling (8). Mutational studies have been performed on IL-2 in an attempt to define the structure/function relationships between IL-2 and the receptor subunits (9–13). Buchli and Ciardelli (14) identified a Gln residue at position 126 of IL-2 that was involved with binding to the $\beta/\gamma$ portion of the high affinity receptor. IL-2 in which Gln-126 was mutated to an Asp residue resulted in an analog with greatly reduced biological activity. Another analog was created in which a Thr residue at position 51 was changed to a Pro residue (15). This analog, despite a decreased binding affinity, increased DNA synthesis of stimulated human peripheral blood lymphocytes to a much greater extent than expected. The authors postulated that the Thr-51 $\rightarrow$ Pro mutation slowed the internalization rate of the ligand-receptor complex, thereby allowing a greater time interval for signaling activation.

The interleukin-2 diphtheria toxin-related fusion protein, DAB$_{389}$ IL-2, is composed of amino acid residues 2–133 of IL-2, genetically fused to the first 386 residues of diphtheria toxin (DT) (16, 17). The fusion toxin is targeted to cells that express the high affinity form of the IL-2 receptor and is internalized by receptor mediated endocytosis. The fusion toxin is processed and the catalytic domain of DT is translocated across the endocytic membrane, into the cell cytosol, where it ADP-ribosylates elongation factor 2, leading to an irreversible inhibition of protein synthesis and subsequent cell death (18–20).

In the present study, we introduced the Q126D and T51P mutations described above, as well as an E106K mutation, into the IL-2 receptor binding domain of DAB$_{389}$ IL-2. We studied the effects of these mutations on cytotoxicity, binding affinity, and kinetics of cytotoxicity. We also created analogous mutations, in which the catalytic domain of DT was mutated to a nontoxic form, so we could study the effects of the IL-2 receptor binding domain on stimulation of DNA synthesis. Our results indicate that the Gln residue is involved with binding affinity and activation of DNA synthesis, and that activation may affect cytotoxicity. The Thr residue appears to affect receptor binding and signaling internalization of the fusion toxin receptor complex. The Gln residue that was mutated does not appear to play a critical role in the IL-2 binding domain of the fusion toxin.

**EXPERIMENTAL PROCEDURES**

Plasmid, Bacterial Strains, and Fusion Toxin Products—A schematic representation of the gene encoding DAB$_{389}$ IL-2, the restriction digest sites used, and the single amino acid residue changes are shown in Fig. 1. The plasmid encoding DAB$_{389}$ IL-2(T439P) was created by a 306-nucleotide MluI to HindIII exchange from the plasmid encoding the T51P mutation in IL-2 (15). This exchange encompassed the region of IL-2 containing the Thr-51 $\rightarrow$ Pro mutation. The plasmids encoding DAB$_{389}$ IL-2(Q514D) and DAB$_{389}$ IL-2(E494K) were created by PCR mutagenesis of the wild type, DAB$_{389}$ IL-2 gene, and cassette exchange encompassing the mutated site (21). In the case of the plasmid encoding DAB$_{389}$ IL-2(Q514D), a 229-nucleotide XbaI to Sall exchange was performed, and for DAB$_{389}$ IL-2(E494K), a 241-nucleotide XbaI to HindIII cassette exchange was performed. The E1495 mutation in the DT catalytic domain was introduced into each of the above mutants by an NcoI to SphI exchange from the gene encoding DA(E1495)B$_{389}$ IL-2. In all cases the constructs were sequenced by the dyeoxy chain termina-
DAB$_{389}$ IL-2 Receptor Interactions

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Expression and Purification of Diphtheria Toxin-related Fusion Proteins—Expression of DAB$_{389}$ IL-2, DAB$_{389}$ IL-2(T439P), DAB$_{389}$ IL-2(E494K), DAB$_{389}$ IL-2-Q514D, and their corresponding E149S catalytic domain mutations, were all under control of the T7 polymerase promoter in derivatives of pet11d (Novagen, Madison WI). The plasmids encoding DAB$_{389}$ IL-2, DA(E149S)B$_{389}$ IL-2, DAB$_{389}$ IL-2(E494K), and DA(E149S)B$_{389}$ IL-2(E494K) were transformed into HMS174 for expression. The bacteria were propagated to an A$_{600}$ = 0.8 in Luria broth (modified Luria broth, with added 25% final concentration of ampicillin and 25% final concentration of chloramphenicol to'A').

Oligonucleotide Synthesis—Oligonucleotides were synthesized on an Applied Biosystems model 391 PCR Mate DNA synthesizer. The oligonucleotides were removed from the columns and deprotected as recommended by Applied Biosystems. The oligonucleotides were vacuum-dried, resuspended in TE buffer, and the concentration determined using absorbance A$_{260}$ readings.

Polymerase Chain Reaction (PCR)—PCR was performed using a PCR reagent kit (Perkin Elmer Corp.). The conditions for PCR were 1 min at 95°C for strand separation, 1 min at 37°C for primer hybridization, and 1 min at 72°C for the polymerase activity. The cycle was repeated 25 times.

Expression and Purification of Diphtheria Toxin-related Fusion Proteins—Expression of DAB$_{389}$ IL-2, DAB$_{389}$ IL-2(T439P), DAB$_{389}$ IL-2(E494K), DAB$_{389}$ IL-2-Q514D, and their corresponding E149S catalytic domain mutations, were all under control of the T7 polymerase promoter in derivatives of pet11d (Novagen, Madison WI).

Table I. IL-2 receptor binding domain of the fusion toxins, are shown in Fig. 1. The mutations in the IL-2 receptor binding domain of the fusion toxins are shown in Table I.

The fusion toxins DAB$_{389}$ IL-2, DA(E149S)B$_{389}$ IL-2, DAB$_{389}$ IL-2(E494K), and DA(E149S)B$_{389}$ IL-2(E494K) were transformed into HMS174 for expression. The bacteria were propagated to an A$_{600}$ = 0.8 in Luria broth (modified Luria broth, with added 25% final concentration of ampicillin and 25% final concentration of chloramphenicol to 'A').

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DAB\textsubscript{389} IL-2 Receptor Interactions

The mutant fusion toxins, with indicated amino acid residue changes in the IL-2 receptor binding domains, and the corresponding changes in IL-2 are listed. The E149S form of the fusion toxins contains the mutation in the catalytic domain which renders the fusion toxin nontoxic.

DA\textsubscript{389} IL-2

DA(E149S)B\textsubscript{389} IL-2

DAB\textsubscript{389} IL-2(E494K)

DA(E149S)B\textsubscript{389} IL-2(E494K)

DAB\textsubscript{389} IL-2(T439P)

DA(E149S)B\textsubscript{389} IL-2(T439P)

DAB\textsubscript{389} IL-2(Q514D)

DA(E149S)B\textsubscript{389} IL-2(Q514D)

DAB\textsubscript{389} IL-2(Q121D)

DAB\textsubscript{389} IL-2

DAB\textsubscript{389} IL-2 No mutation

DA(E149S)B\textsubscript{389} IL-2(E494K)

DA(E149S)B\textsubscript{389} IL-2(T439P)

DA(E149S)B\textsubscript{389} IL-2(Q514D)

DA(E149S)B\textsubscript{389} IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

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DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

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DAB389 IL-2(E494K)

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DAB389 IL-2(Q514D)

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DAB389 IL-2(E494K)

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DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

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DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

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DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

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DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2

DAB389 IL-2(E494K)

DAB389 IL-2(T439P)

DAB389 IL-2(Q514D)

DAB389 IL-2(Q121D)

DAB389 IL-2

DAB389 IL-2
IL-2(T439P) and DAB_{389} IL-2(Q514D) exhibited reduced rates of intoxication (Fig. 6).

**DISCUSSION**

Studies of the relationships between IL-2 and its receptors are important to identify the various functions of IL-2, as well as the structural elements involved with these functions. This information is required for the design of analogs with expanded therapeutic applications. In the present work we use the IL-2-directed fusion toxin, DAB_{389} IL-2, to further study some amino acid residues previously identified as involved in the processing of IL-2 and its receptors. The purpose of this work was to expand on the knowledge already obtained for these residues, to ascertain how these residues affect the function of the fusion toxin, and to demonstrate the potential of DAB_{389} IL-2 as a useful agent for the study of ligand-receptor interactions.

Buchli and Ciardelli (14) created an IL-2 analog in which a Gln residue at position 126 of IL-2 was changed to an Asp residue. Their results indicated that the Asp-126 mutant stimulated \[^3\text{H}\]thymidine incorporation in human T-lymphocytes and CTLL-2 cells to a lesser degree than wild type IL-2. The binding affinity of the D126 mutant was greatly decreased, and the loss was due to a disruption of the \(\beta\gamma\) receptor subunit interaction. The authors postulated that cross-linking of the \(\beta\gamma\) receptor subunits is the likely signaling event for activity of IL-2, and that Gln-126 is involved with binding and cross-linking the subunits, either as a contact position or allosterically.

We constructed and studied DAB_{389} IL-2(Q514D), which contains the analogous mutation in the IL-2 receptor binding domain of DAB_{389} IL-2. For comparison, we also studied DAB_{389} IL-2(E494K), a form of DAB_{389} IL-2 containing a mutation in the IL-2 binding domain that we already knew exerted minimal effects on cytotoxicity. DAB_{389} IL-2(Q514D) was 2000-fold less cytotoxic than wild type DAB_{389} IL-2 and possessed a decreased binding affinity, and the corresponding protein with the catalytic domain mutation did not stimulate \[^3\text{H}\]thymidine incorporation. The cytotoxicity kinetic assays indicate that DAB_{389} IL-2(Q514D) inhibited protein synthesis at a slower rate than wild type DAB_{389} IL-2. The rate of inhibition reflects the rate of binding and toxin entry into the cell cytosol. These results are all consistent with the findings for the Gln-126 residue of IL-2. DAB_{389} IL-2(E494K) was 7-fold less cytotoxic than DAB_{389} IL-2, probably as a direct result of the 8-fold decrease in binding affinity. DAB_{389} IL-2(T439P) stimulated \[^3\text{H}\]thymidine incorporation, although not as much as wild type, DA(E149S)B389 IL-2. The effects imposed by the Gln-494 mutation to a Lys probably result from structural changes in the IL-2 binding domain of the fusion toxin, leading to the decrease in binding affinity.

Chang et al. (15) discovered that mutating Thr-51 of IL-2 to a Pro residue resulted in an IL-2 analog with a decreased binding affinity, but the corresponding loss in stimulation of DNA synthesis was much lower than expected. They postulated that either the Thr-51 \(\rightarrow\) Pro mutation resulted in a conformational change that partially mimicked a change required for IL-2 to facilitate the \(\beta\gamma\) subunit cross-linking necessary for signaling activation, or the Thr-51 \(\rightarrow\) Pro mutation slowed the internalization rate for the ligand-bound receptor complex, thereby allowing a greater time for the signaling interval. The corresponding residue in the IL-2 receptor binding domain of DAB_{389} IL-2 was mutated to create DAB_{389}
DAB\textsubscript{389} IL-2(T439P). DAB\textsubscript{389} IL-2(T439P) was 300-fold less cytotoxic than wild type and possessed a decreased binding affinity, but still stimulated DNA synthesis as well as the control mutation, DAB\textsubscript{389} IL-2(E494K). SDS-polyacrylamide gel electrophoresis of DAB\textsubscript{389} IL-2(T439P) (Fig. 2, lane 3) shows this protein was subject to breakdown after freezing and thawing, probably due to conformational effects the Thr-439 → Pro mutation had on the protein. (DAB\textsubscript{389} IL-2(T439P) was originally isolated as full-length protein, as shown by gel electrophoretic analysis performed during the purification procedures; data not shown.)

DAB\textsubscript{389} IL-2(T439P) and DAB\textsubscript{389} IL-2(Q514D) both possessed poor binding affinities, but DAB\textsubscript{389} IL-2(T439P) was 10-fold more cytotoxic. The fact that DAB\textsubscript{389} IL-2(T439P) stimulated DNA synthesis and DAB\textsubscript{389} IL-2(Q514D) did not lead us to speculate that the difference in stimulatory effect may account for the difference in cytotoxicity, i.e. stimulation of DNA synthesis leads to enhancement of cytotoxicity. The cytotoxicity kinetics assay for DAB\textsubscript{389} IL-2(T439P) showed that this mutant fusion toxin, like DAB\textsubscript{389} IL-2(Q514D), possessed a decreased rate of cytotoxicity compared to the wild type and DAB\textsubscript{389} IL-2(E494K). This leads to the conclusion that the Thr-439 → Pro mutation in the IL-2 receptor binding domain of DAB\textsubscript{389} IL-2, and the corresponding Thr51 to Pro mutation in IL-2, exerted greater than expected stimulatory effects at least partially due to a decreased rate of internalization.

The results from these studies confirm and expand on the previous findings for studies performed with IL-2. The Asp residue at position 126 of IL-2 is involved with binding of IL-2 to its receptors. The rate of internalization of the IL-2-receptor complex is decreased and, possibly due to a decrease in βγ cross-linking, signaling stimulation of DNA synthesis is decreased. Additionally, mutation of this residue in the IL-2 binding domain of the fusion toxin DAB\textsubscript{389} IL-2 decreases cytotoxicity to a greater degree than expected, indicating that the loss in stimulation of DNA synthesis, affects cytotoxicity. It appears that the stimulatory effect of the IL-2 binding domain on DNA synthesis enhances cytotoxicity. The Thr residue at position 51 of IL-2 is involved with binding, and this effect may be conformational. The rate of ligand-receptor internalization is decreased when this residue is changed to a Pro, and an increase in DNA stimulation occurs. This effect results in a greater than expected cytotoxicity when the corresponding residue in the IL-2 binding domain of DAB\textsubscript{389} IL-2 is mutated.

REFERENCES

1. Gillis, S., Fern, M. M., Ou, W., and Smith, K. A. (1978) Immunology 120, 2027
2. Beckner, S. W., and Farrar, W. L. (1987) Biochem. Biophys. Res. Commun. 145, 176–182
3. Mills, G. B., Cragoe, E. J., Jr., Gefand, E. W., and Grinstein, S. (1985) J. Biol. Chem. 260, 12500–12507
4. Ishii, T., Takeshita, T., Numata, J., and Sugamura, K. (1988) J. Immunol. 141, 174–179
5. Rayhet, E. J., Fields, T. J., Albright, J. W., Diamantstein, T., and Hughes, J. P. (1988) Biochem. J. 249, 333–338
6. Farrar, W. L., and Anderson, W. B. (1985) Nature 315, 233–235
7. Landgraf, B. E., Goldman, B., Williams, D. P., Murphy, J. H., Sana, T. R., Smith, K. A., and Ciardelli, T. L. (1992) J. Biol. Chem. 267, 18511–18519
8. Takeshita, T., Asao, H., Ohtani, K., Ishii, N., Kumaki, S., Tanaka, N., Munakata, H., Nakamura, M., and Sugamura, K. (1992) Science 257, 379–382
9. Berndt, W. G., Chang, D. Z., Smith, K. A., and Ciardelli, T. L. (1994) Biochemistry 33, 6571–6577
10. Berndt, W. G., and Ciardelli, T. L. (1992) Interleukin-2, pp. 12–28, Blackwell Scientific Publications, Oxford
11. Zurawski, S. M., and Zurawski, G. (1992) EMBO J. 11, 3905–3910
12. Collins, L., Tsien, W. H., Seals, C., Hakimi, J., Weber, D., Bailon, P., Hoskins, J., Greene, W. C., Toome, V., and J. U., G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7709–7713
13. Suave, K., Nachman, M., Spence, C., Bailon, P., Campbell, E., Tsien, W. H., Kondas, J. A., Hakimi, J., and J. G., G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4636–4640
14. Buchli, P., and Ciardelli, T. (1993) Arch. Biochem. Biophys. 207, 411–415
15. Chang, D. Z., Tisayco, M. L., and Ciardelli, T. L. (1995) Mol. Pharmacol. 47, 206–211
16. Williams, D. P., Parker, K., Bacha, P., Bishai, B., Borowski, M., Genbauffe, F., Strom, T. B., and Murphy, J. H. (1987) Protein Eng. 1, 493–498
17. Williams, D. P., Snider, C. E., Strom, T. B., and Murphy, J. R. (1990) J. Biol. Chem. 265, 11885–11899
18. Badia, P., Williams, D. P., Waters, C., Williams, J. M., Murphy, J. R., and Strom, T. B. (1988) J. Exp. Med. 167, 612–622
19. Waters, C. A., Schnike, P. A., Snider, C. E., Itoh, K., Smith, K. A., Nichols, J. C., Strom, T. B., and Murphy, J. R. (1990) Eur. J. Immunol. 20, 785–791
20. Williams, D. P., Wen, Z., Watson, R. S., Bailon, P., Strom, T. B., and Murphy, J. R. (1990) J. Biol. Chem. 265, 20673–20677
21. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1991) Current Protocols in Molecular Biology, Vol. 1, pp. 8.5.7–8.5.9, Suppl. 15, Greene Publishing Associated/John Wiley & Sons, Inc., New York
22. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
23. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, pp. 90–91, 468, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. van der Spek, J. C., Mindell, J. A., Finkelstein, A., and Murphy, J. R. (1993) J. Biol. Chem. 268, 12077–12082
25. Wang, H. M., and Smith, K. A. (1987) J. Exp. Med. 166, 1055–1069