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Characterization of a CD40-Dominant Inhibitory Receptor Mutant

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CD40 is an important mediator of immune and inflammatory responses. It is a costimulatory molecule for B cell proliferation and survival. Blockade of CD40 has been shown to induce tolerance and its role in other pathogenic conditions has led to the proposal that CD40 inhibition could be valuable therapeutically. As a first step to this end, we have characterized a CD40-dominant negative receptor. This inhibitory mutant lacks the identified CD40 signaling domains. It inhibits both cotransfected and endogenous CD40 activation of NF-κB. This mutant is specific, as it does not affect TNF or latent membrane protein 1 signaling. Its potential usefulness is illustrated by its ability to inhibit the CD40 ligand-stimulated increases of HLA and CD54 expression, molecules involved in Ag recognition and lymphocyte recruitment leading to organ rejection. The inhibitory mutant has no TNFR-associated factor 2-binding capabilities and inhibits the recruitment of TNFR-associated factor 2 to the CD40 signaling complex after stimulation. These studies show that the CD40 inhibitory receptor molecule is effective, specific, and useful both for research and potentially as a clinical tool. And furthermore, it is likely that similar dominant inhibitory receptors can be generated for all of the members of the TNFR superfamily. The Journal of Immunology, 2001, 167: 6388–6393.

The CD40 is a member of the TNFR superfamily of cytokine receptors. It binds CD40 ligand (CD40L or CD154) during germinal center formation and provides important signals for B cell activation and survival. It is also involved in dendritic cell-B cell interactions. CD40 is expressed on a range of other cell types where it plays diverse roles including inhibition of cell growth and the induction of proinflammatory cytokines (for review, see Ref. 1).

CD40 signaling pathways and their effects on cellular phenotype have been intensively studied. CD40 consists of an extracellular CD40L-binding domain and an intracellular signaling domain with two distinct functional regions, one of which binds TNFR-associated factor (TRAF) 6 (2) and the other binds TRAF1, TRAF2, and TRAF3 molecules (3–5). Ligation of CD40 leads to the association of TRAF complexes and activation of various kinase cascades which in turn activate transcription factors, such as NF-κB (6–8) and AP-1 (9). Deletion studies of the regions further demonstrated the importance of the signaling molecules and their role in transcriptional activation functions (10, 11). CD40 signaling results in the expression of adhesion molecules (12), cytokines (13), and apoptotic mediators (14), which are associated with pathogenic processes of chronic inflammatory diseases such as autoimmune disease and graft-vs-host disease (1, 15).

Despite the importance of CD40 in numerous pathogenic conditions, few tools to specifically inhibit CD40 function are available. Abs to CD40L have been used to block CD40-CD40L interaction and have been shown to induce tolerance in transplant models. However, administration of Abs as therapy can cause cytotoxic problems (16, 17). This study was initiated to investigate the dominant inhibitory properties of a CD40 molecule in which the signaling domains have been mutated or removed. We have demonstrated the effectiveness, specificity, and functionality of the CD40-dominant inhibitory mutant. Therefore, we believe that the mutant will be useful for characterizing cell-specific effects of CD40 and has also the potential to be used as a therapeutic tool.

Materials and Methods

Gene transfection

For transient expression, 0.5–1 × 10⁷ cells from a suspension culture were transfected by electroporation using a Bio-Rad Gene pulser II electroporator (Bio-Rad, Hercules, CA) at 280 V and 950 μF at room temperature in 500 μl of growth medium. The cells were reseeded in 5 ml of fresh growth medium and were then incubated under normal conditions. Transfection efficiency ranged from 10 to 20% for EL-4L (EBV-negative Burkitt lymphoma cell line, Ref. 18) and DG75 (EBV-negative Burkitt lymphoma cell line Ref. 19), and up to 40–50% for Jurkat (T cell line, Ref. 20), as assessed by cotransfection with the EGFP-C1 expression vector and flow cytometry analysis. At 24 h after transfection, cells were stimulated with CD40L-expressing fibroblasts (21), which were irradiated (5000 rad) and incubated for another 24 h. As a control for CD40L-specific effect, incubation of transfected DG75 cells with nontransformed fibroblasts (22) had no effect on NF-κB induction (data not shown).

Assay for NF-κB activity

The activity of the NF-κB reporter plasmid was measured 24 h posttransfection. Cells were washed twice in PBS and lysed in 150 μl of lysis buffer containing 100 mM HEPES (pH 8.0), 2 mM magnesium chloride, 5 mM DTT, and 2% Triton X-100. Luciferase activity in 50 μl of clarified lysate was analyzed in a Berthold LB9501 luminometer following injection of 100 μl of 0.5 mM luciferin (USB, Cleveland, OH) dissolved in luciferin assay reagent (30 mM glycylglycin (pH 7.9), 1 mM MgCl₂, 0.1 mM...
Analysis of cell surface protein expression

The induction of CD40, CD54, and HLA proteins in transfected cells was assayed by immunofluorescence staining of viable cells by flow cytometry using a BD Biosciences FACSCalibur. Briefly, at 48 h posttransfection the cells were washed and stained with a PE-conjugated mAb to either human CD54 (MCA675PE; Serotec, Oxford, U.K.) or CD40 (MCA1590PE; Serotec) at 4°C for 60 min. For HLA staining, we used W632 supernatant at 4°C for 60 min followed by mouse IgRPE (R0439; DAKO, Glostrup, Denmark) at 4°C for 45 min. The transfected population was marked by the expression of cotransfected EGFP-C1 plasmid and gated for analysis of surface protein staining.

Immunoprecipitations and Western blotting

For each immunoprecipitation, 15 × 10^6 cells of the Jurkat or DG75 cell line in 0.5 ml of growth medium were electroporated at 280 V and 950 μF with either latent membrane protein 1 (LMP1; control), CD40 wild-type (wt), or CD40 delA-expressing plasmid DNA along with 6 μg of TRAF2-expressing plasmid. At 24 h posttransfection, half of the cells were incubated with CD40L-expressing fibroblast as described earlier. At 48 h posttransfection the cells were washed twice with PBS, then lysed for 45 min on ice in 800 μl of Nonidet P-40 lysis buffer (0.5% Nonidet P-40, 50 mM HEPES buffer, 0.25 M NaCl, and 2 mM EDTA), to which a 5% protease inhibitor mixture (P8340; Sigma-Aldrich, St. Louis, MO) was added before use. The lysates were clarified by centrifugation at 13,000 × g for 5 min, and the soluble fraction was then preclarified for 4 h with 20 μl of Sepharose-4B protein G, to which 1 μg of control Ab had been covalently cross-linked. The preclarified lysate was then incubated overnight with 20 μl of Sepharose-protein G, to which 1 μg of CD40 rabbit Ab (sc975; Santa Cruz Biotechnology, Santa Cruz, CA) or 1 μg of CD40 mouse Ab (815002; Ancell, Bayport, MN) or a mixture of the LMP1 Abs CS.3 (0.5 μg) and CS.4 (0.5 μg) had been covalently cross-linked. The immunoprecipitates were washed three times and eluted by boiling in 40 μl of SDS gel sample buffer. Half of the eluate was separated by SDS-PAGE and analyzed for TRAF2 expression by Western blotting using a TRAF2 rabbit polyclonal Ab (sc876; Santa Cruz Biotechnology).

Results

The CD40 mutant, CD40 delA, is nonfunctional and can act as a dominant negative

This project was initiated to test the hypothesis that a CD40 molecule, which lacked signaling properties, could be used as a dominant inhibitory molecule. The CD40 mutant used in this study, CD40 delA, is mutated and deleted at the critical sequences for binding the TRAF signaling molecules (2, 4, 5, 23). This mutant therefore lacks the ability to bind any signaling molecules (10, 24) and was recently used to identify the region involved in CD40-induced apoptosis (11). The features of the CD40 delA mutant with the deleted TRAF6-binding region (del 219–239) and the point-mutated second TRAF-binding domain (T254A) (11) are shown in Fig. 1a.

CD40 induces many different signal-transducing pathways, such as mitogen-activated protein kinase pathways and the NF-κB pathway, which are important for the regulation of cell survival and apoptosis (25). However, one of the most important signaling cascades involved in CD40 signaling is the activation of the transcription factor NF-κB. Hence, our first step was to characterize the ability of CD40 delA to activate a NF-κB luciferase reporter in Jurkat cells, a T lymphoblastoid cell line negative for CD40. Fig. 1b shows that while expression of wt CD40 efficiently induces NF-κB activity, CD40 delA caused only a very small increase in luciferase activity. This suggested that CD40 delA is a good candidate for a dominant inhibitory CD40 molecule. We tested this possibility by transient transfection of Jurkat cells with increasing amounts of CD40 delA and a constant dose of CD40-expressing plasmid (Fig. 1c). The results show a dose-dependent inhibition of CD40-induced NF-κB activation by CD40 delA with a maximal down-regulation of NF-κB activation below 10% of CD40 activity (Fig. 1c).

It should also be noted, that transfection of other plasmids did not nonspecifically inhibit CD40-induced NF-κB activation (see Fig. 3). These results clearly demonstrate that CD40 delA is able to effectively inhibit CD40-induced NF-κB activation and has therefore the potential to act as a dominant negative inhibitor for the CD40 receptor.
The CD40<sup>delA</sup> mutant is an effective inhibitor of endogenous CD40 signaling

CD40 signaling is important for B cell activation. The effect of CD40<sup>delA</sup> on endogenous CD40 was investigated in B cells that express CD40. There are many B cell lines described which respond to CD40L-induced stimulation in terms of NF-κB activation and induction of cell surface molecules (26). We chose the DG75 and Eli-BL B cell lines, both of which can be transfected and effectively stimulated using CD40L-expressing fibroblasts. First of all, we analyzed the expression levels of CD40 on cells transfected with control plasmid and CD40<sup>delA</sup>-expressing plasmid (Fig. 2, a and b). For this purpose, DG75 cells were transfected with different amounts of CD40<sup>delA</sup>-expressing plasmid along with a fixed amount of green fluorescence protein (GFP)-expressing plasmid. At 48 h posttransfection, the cells were stained for CD40 and the positive transfected population was analyzed by flow cytometry.

Transfection of 5 μg of CD40<sup>delA</sup> results in a three times higher mean fluorescence intensity compared with basal CD40 levels in those cells, and transfection with high amounts of the CD40<sup>delA</sup> (such as 20 μg) shows a 6-fold higher CD40 mean fluorescence intensity than control transfected cells (Fig. 2b).

After determining the expression levels of transfected and endogenous CD40, we analyzed the NF-κB activation by CD40L-induced CD40 stimulation in the transfected cells expressing CD40<sup>delA</sup>. For this purpose, DG75 and Eli-BL cells were transfected with a range of concentrations of CD40<sup>delA</sup>-expressing plasmid (0–20 μg) along with NF-κB luciferase reporter plasmid, and the luciferase activity in the CD40L-stimulated cells was measured (Fig. 2, c and d). The results show that DG75 cells have a much higher basal NF-κB level than Eli-BL cells and, more importantly, that CD40<sup>delA</sup> is able to reduce the luciferase activity in both stimulated cell lines down to basal NF-κB activity levels. This demonstrates that CD40<sup>delA</sup> effectively inhibits endogenous CD40-induced NF-κB activation in a dose-dependent manner down to the basal NF-κB activity levels in CD40 responder cell lines.

CD40<sup>delA</sup> is specific for CD40-induced signaling pathways

CD40 shares intracellular signal-transducing molecules and pathways with other members of the TNFR superfamily. Our strategy, using a dominant negative CD40 receptor molecule, should allow inhibition of CD40/CD40L signaling without interfering with other receptors using the same signal transduction pathways. To

**FIGURE 2.** Inhibition of endogenous CD40 signaling by CD40<sup>delA</sup>. DG75, or Eli-BL cells were transfected with increasing amounts of CD40<sup>delA</sup> plasmid along with a constant amount (1 μg) of GFP-expressing plasmid. After 8 h posttransfection, half of the cells were incubated with irradiated (5000 rad) CD40L-expressing fibroblasts until they were assayed. The controls only transfected with empty vector and GFP, lacking the CD40<sup>delA</sup> plasmid, were always treated in the same way. a, CD40 expression was determined in nonstimulated DG75 cells 48 h posttransfection by fluorescence staining and flow cytometry (FACS). The histogram shows the number of CD40-positive cells either transfected with control vector (dark gray) or with 5 μg of CD40<sup>delA</sup> (light gray) relative to basal levels (black) of the GFP-transfected cell population. b, CD40 expression levels (mean fluorescence intensity, MFI) were determined in nonstimulated DG75 cells transfected with a range of 0–20 μg of CD40<sup>delA</sup> plasmid. The results shown are representative of at least three independent experiments. c and d, NF-κB luciferase activity was measured in CD40L-stimulated (■) and nonstimulated (□) responder cell lines. DG75 (c) and Eli-BL (d) cells were transfected with NF-κB reporter plasmid (3 μg) and with a range of 0–20 μg or 0–8 μg of CD40<sup>delA</sup> plasmid, respectively. The plasmid concentration was kept constant by adding empty vector to the final concentration of 20 or 8 μg. Unstimulated cells and cells exposed to control fibroblasts showed no NF-κB induction. The results shown are representative of at least three independent experiments.
test this hypothesis, we examined the effect of CD40<sup>delA</sup> upon NF-κB activation by two related mechanisms, the TNFR/TNF-α and the LMP1 of the EBV. Both molecules have been shown to induce NF-κB transcriptional activation after stimulation (6, 27) and are able to bind several cytosolic signaling factors, including the TRAFs (28–30). Given the similarity among CD40, TNF-α, and LMP1 signaling, we tested the specificity of the CD40<sup>delA</sup> mutant in these related systems using a NF-κB luciferase reporter assay and comparing CD40-induced NF-κB activation with TNF-α and LMP1. With this system, we also analyzed the effects of a dominant negative form of the LMP1 (LMP1dn) (31), which has lost its signaling molecule binding function by point-mutating amino acids at positions 204, 206, 208, and 384 in the two so-called C-terminal activator regions. The experiments were conducted as transient transfection reporter assays, analyzing NF-κB luciferase activity in Jurkat cells transfected with CD40<sup>delA</sup> or LMP1dn-expressing plasmid, which were either stimulated with TNF-α or cotransfected with wt LMP1 or CD40, respectively (Fig. 3). The results shown here clearly demonstrate that CD40<sup>delA</sup> does not interfere with the ability of either TNF-α or LMP1 to activate the NF-κB signaling pathway in the transfected cells. Furthermore, the LMP1-dominant negative mutant, which was designed using a similar strategy to CD40<sup>delA</sup>, does not inhibit TNF-α or CD40-induced NF-κB. Thus, we conclude that the CD40<sup>delA</sup> and LMP1dn mutants are specific inhibitors and do not interfere with the signaling capabilities of the other receptors. This implies a range of applications for specific dominant negative receptor molecules and should lead to the development of other TNFR-based dominant negative mutants.

**CD40<sup>delA</sup> inhibits CD40-induced phenotypical changes**

NF-κB is a major effector molecule involved in CD40 signaling and is responsible for many cell surface changes due to CD40 ligation, such as expression of CD54 and HLA, which are involved in immune responses (1, 32, 33). HLA, for example, is one of the most important molecules involved in immune responses directed against alloantigens on grafts; thus, inhibition of CD40-mediated up-regulation by CD40<sup>delA</sup> could have great potential. In the case of CD54 it is known that, in acute rejection, CD40 stimulation and is responsible for many cell surface changes due to CD40 ligation, such as expression of CD54 and HLA, which are involved in immune responses (1, 32, 33). HLA, for example, is one of the most important molecules involved in immune responses directed against alloantigens on grafts; thus, inhibition of CD40-mediated up-regulation by CD40<sup>delA</sup> could have great potential. In the case of CD54 it is known that, in acute rejection, CD40 stimulation and CD54 expression are important mediators of lymphocyte recruit-

**FIGURE 3.** Specificity of the CD40<sup>delA</sup> and LMP1dn mutants. Jurkat cells were transfected with empty vector, LMP1dn (3 μg), or CD40<sup>delA</sup> plasmid (5 μg) along with NF-κB reporter plasmid (3 μg) and either wt LMP1 (3 μg) or wt CD40 (3 μg) constructs. The cells were incubated overnight to allow protein expression and, in the case of the TNF-α stimulation (left panel), the cells were treated for 6 h with TNF-α (10 ng/ml) before luciferase activity was measured. The data, shown in relation to stimulated control transfectants (set to 100%), representing a mean value of 1856 ± 324 luciferase units for TNF-α (+ column in left panel), 2353 ± 782 U for LMP1 (+ column in middle panel), and 4128 ± 444 U for CD40 (+ column in right panel), are representative of at least three independent experiments.

**FIGURE 4.** Analysis of the biological function of CD40<sup>delA</sup>. DG75 cells were transfected with increasing amounts of CD40<sup>delA</sup> plasmid along with GFP-expressing plasmid (1 μg). At 24 h posttransfection, half of the cells were incubated with irradiated (5000 rad) CD40L-expressing fibroblasts for another 24 h before analyzing surface marker expression by fluorescence staining and flow cytometry (FACS). The controls lacking the CD40<sup>delA</sup> plasmid were treated in the same way. a, CD54 mean fluorescence intensity (MFI) of GFP-positive cells in nonstimulated (No Ligand) and stimulated (+ CD40 Ligand) cultures. The results shown are representative of at least three independent experiments. b, HLA mean fluorescence intensity (MFI) of GFP-positive cells in the nonstimulated (− Ligand) and stimulated (+ Ligand) cultures. The results shown are representative of at least three independent experiments.
mutated for the TRAF binding sites it should be unable to bind TRAF2. To investigate this possibility, coimmunoprecipitation experiments were conducted to analyze the binding of TRAF2 to CD40 and CD40<sup>delA</sup> and to determine whether CD40<sup>delA</sup> could inhibit the recruitment of TRAF2 to wt CD40. For this purpose, Jurkat and DG75 cells were transfected with LMP1, CD40 wt, CD40<sup>delA</sup>, or both CD40wt- and CD40<sup>delA</sup>-expressing plasmids in the presence of TRAF2 (Fig. 5). The cells were left for 24 h to allow expression before they were stimulated with CD40L for another 24 h. The cells were then lysed and the lysates were pre-cleared with Sepharose-protein G. LMP1 was immunoprecipitated with the Abs CS.3 and CS.4 as a positive control for a protein that binds TRAF2. CD40 was immunoprecipitated with either a specific polyclonal rabbit CD40 Ab (Fig. 5a) or with a specific monoclonal mouse CD40 Ab (Fig. 5b). The immunoprecipitates were resolved by SDS-PAGE and the presence of TRAF2 was detected by Western blotting. The results from a representative experiment in Jurkat cells are shown in Fig. 5a (top panel). It shows that TRAF2 can be coimmunoprecipitated with both LMP1 and CD40. However, CD40<sup>delA</sup>, which lacks the TRAF binding site, did not coimmunoprecipitate TRAF2. In the cells cotransfected with wt CD40 and CD40<sup>delA</sup>, coimmunoprecipitated TRAF2 could not be detected. Similar results were obtained in DG75 cells (Fig. 5b). However, TRAF2 binding was only observed after stimulation of the cells with CD40L. These results support the hypothesis that CD40<sup>delA</sup> inhibits the binding of TRAF2 to CD40 via a mechanism whereby CD40<sup>delA</sup> binds to wt CD40, preventing TRAF2 binding; thus inhibiting CD40-induced signal transducing pathways. These data provide a mechanism for inhibition of signaling by CD40<sup>delA</sup>, which result in the inhibition of phenotypical changes.

**Discussion**

This study describes the characterization of a dominant inhibitory CD40 molecule, CD40<sup>delA</sup>. This CD40 mutant is effective and specific for CD40 signaling, inhibiting the activation of the transcription factor, NF-κB, and the induction of gene expression changes. Our choice of CD40<sup>delA</sup> as a dominant inhibitory molecule was inspired by our previous experience with the EBV-encoded protein LMP1, which functions like a constitutive member of the TNF family. We have generated a dominant inhibitory form of LMP1 (31) by mutating the TRAF binding sites in the molecule. The coexpression of dominant inhibitory LMP1 prevents the binding of TRAF2 to wt LMP1. CD40<sup>delA</sup> lacks the ability to bind any signaling molecules (10, 24). Our experiments showed that CD40<sup>delA</sup> could prevent coimmunoprecipitation of TRAF2 with wt CD40, suggesting that dominant inhibitory CD40 and LMP1 share a similar mechanism. TRAF2 binding requires a conserved binding motif (35) and receptor oligomerization (36, 37). Crystalllographic analysis shows that the trimeric receptor complex recognizes a TRAF trimer (38). Our proposed mechanism is that incorporation of CD40<sup>delA</sup> into the CD40 receptor complex prevents the binding of TRAFs and thus the downstream signals. The observation that CD40<sup>delA</sup> can also inhibit endogenous CD40 signaling is a substantial advance and demonstrates the effectiveness of this approach.

This study combined with our work on LMP1 has led to a hypothesis that all members of the TNFR superfamily can be inhibited by such an approach. A study of lymphoproliferative syndromes identified dominant interfering Fas mutations that are similar to the ones that we have generated for LMP1 and CD40 (39). Furthermore, a receptor-based mutant has also been generated for TNF (40), although this was a receptor truncation, which may have less efficiency than receptors with smaller mutations. The generation of receptor-based mutants for LMP1, CD40, Fas, and TNF implies that all members of the TNFR superfamily may be exploited in this way.

One of the major benefits of a receptor-based dominant inhibitor approach is specificity. We have shown that the introduction of CD40<sup>delA</sup> does not affect either TNF signaling through its endogenous receptor or cotransfected LMP1 signaling while effectively inhibiting coexpressed CD40. Similar specificity was observed for the dominant negative LMP1. This approach has the potential for more selective inhibition than that provided by the use of dominant inhibitory forms of shared signaling molecules, such as TRAFs, TNFR-associated death domain, or IκB, the inhibitor of NF-κB. Although they are useful, these mutants lack selectivity since these signaling molecules are shared by many different cell stimuli.

NF-κB is one of the most important signaling pathways stimulated by CD40. CD40<sup>delA</sup> effectively inhibits NF-κB activation. We chose not to test each individual CD40-stimulated pathway but instead to focus on gene expression changes. CD40 increases the expression of MHC class I and ICAM-1 (CD54), which are known to be important for immune responses (1, 32, 33). CD40<sup>delA</sup> inhibits the induction of both of these proteins. ICAM-1 is particularly interesting, as maximal induction of ICAM-1 by LMP1 has been shown to involve, as yet, uncharacterized signaling pathways (41). Although we have not investigated the pathways that CD40 utilizes for the regulation of ICAM-1, the parallels between LMP1 and CD40 signaling suggests that all their pathways may be shared. Thus, the inhibition of ICAM-1 and HLA induction by CD40<sup>delA</sup> suggests that pathways beyond NF-κB activation are effectively inhibited.

CD40 induction of HLA and ICAM-1 plays a role in graft rejection (34). Blockade of CD40 has been shown to induce tolerance (42–44). The inhibition of these important molecules by the CD40<sup>delA</sup> mutant suggests that it may be as effective as Ab blockade. However, it is important to verify the use of CD40<sup>delA</sup> in an animal model. Experiments to investigate the in vivo applicability of CD40<sup>delA</sup> to inhibit immune responses will be conducted in a rodent model using a viral-based gene delivery used previously (45). The effectiveness of the dominant interfering Fas in a transgenic model provides some in vivo evidence for this approach.

**FIGURE 5.** Analysis of TRAF2 recruitment of CD40<sup>delA</sup>. To analyze the TRAF2 binding abilities, Jurkat (a) and DG75 (b) cells were transfected with empty vector, LMP1 (positive control), CD40 (wt), CD40<sup>delA</sup>, or CD40 along with CD40<sup>delA</sup> (wt + delA) together with TRAF2-expressing plasmid (6 μg). At 24 h posttransfection, the cells were incubated with irradiated (5000 rad) CD40L-expressing fibroblasts for another 24 h. The cells were lysed and samples for input lysates were taken. The precleared extracts were incubated with cross-linked protein G-Sepharose using either LMP1 (positive control) or rabbit polyclonal CD40 (a) or mouse monoclonal CD40 (b) Abs. After 12 h of incubation, the beads were washed and the proteins were eluted by boiling in gel sample buffer. The proteins were separated using SDS-PAGE and the presence of TRAF2 was investigated by Western blotting probing with rabbit antisera to TRAF2.
(46). The validation of the CD40ΔdelA mutant in vivo is the next step toward the exploitation of this molecule in the clinic.

In conclusion, the functionality, specificity, and efficiency of CD40ΔdelA shows that this mutant could serve as a dominant negative tool to inhibit CD40 signaling involved in pathogenic conditions. The potential of the CD40ΔdelA mutant for therapy is particularly interesting in light of the questions about the application of anti-CD40L Ab treatment to humans and the lack of synthetic inhibitors for the CD40-CD40L interaction (1, 43). Thus, dominant negative receptor mutants may be useful tools for additional therapeutic strategies.

Acknowledgments

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References