Differential Induction of Cytokine Genes and Activation of Mitogen-Activated Protein Kinase Family by Soluble CD40 Ligand and TNF in a Human Follicular Dendritic Cell Line

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*J Immunol* 1999; 163:631-638; ;
http://www.jimmunol.org/content/163/2/631

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Follicular dendritic cells (FDC) play crucial roles in germinal center (GC) formation and differentiation of GC B cells. Many aspects of FDC function are influenced by contact with B or T cells, and by cytokines produced in the GC, which involve stimulation of CD40 and TNF-α receptors on FDC. In this study, using an established FDC line, HK cells, we compared the effects of CD40 and TNF receptor triggering on cytokine induction and activation of mitogen-activated protein kinase family. We show that HK cells spontaneously produced IL-6, M-CSF, and G-CSF mRNA. While TNF strongly induced IL-6 mRNA, its expression was not affected by sCD40L treatment, differing from the strong IL-6 induction in other cell types upon CD40 stimulation. In addition, sCD40L treatment resulted in activation of extracellular signal-related kinase 1 and 2 (ERK1/2) and p38 without significant increase in c-Jun N-terminal kinase (JNK) activity. Lack of JNK activation differs in that most B cells respond to CD40 stimulation by inducing JNK activity strongly, suggesting distinct characteristics of CD40 signaling in FDC. Compared with the effects of sCD40L, TNF was capable of inducing JNK activity in addition to the activation of ERK1/2 and p38. Furthermore, the proximal signaling elements activated by TNF differed from those activated by sCD40L, in that TNF did not require PMA-sensitive protein kinase C isoforms in the activation of ERK and p38, whereas sCD40L did. However, signals activated by these stimuli converged on cytokine gene expression in a synergistic manner, which may have implication in augmenting FDC function during GC reaction. The Journal of Immunology, 1999, 163: 631–638.
MAPK. While TNF induced c-Jun N-terminal kinase (JNK) activity strongly, sCD40L activated it only marginally. In addition, the requirement for proximal signaling elements leading to ERK and p38 activation and to cytokine gene induction by these two molecules was distinct from each other, i.e., sCD40L requires phorbol ester-sensitive protein kinase C (PKC) isoforms, whereas TNF does not. This differential usage of PKC activity in sCD40L and TNF-activated signals is further substantiated by the observation that these molecules cooperatively enhanced M-CSF and G-CSF gene expression in HK cells, suggesting a synergism in FDC activation during GC reactions.

Materials and Methods

Cell cultures

The cell culture media were purchased from Life Technologies (Rockville, MD). An established FDC line (HK cells) (3) obtained from Dr. Y. S. Choi (Alton Ochsner Medical Foundation, New Orleans, LA), was used at passages 14–16. HK cells were grown in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Human fetal lung fibroblasts, LF-1 cells, and embryonic kidney cell line 293T were maintained in DMEM supplemented with 10% FCS.

Reagents

Rabbit polyclonal anti-CD40L Ab (C-20) corresponding to an amino acid sequence mapping at the C terminus of human CD40L was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and the anti-CD40 mAb G28-5 from the American Type Culture Collection (Manassas, VA). The rabbit anti-phospho-ERK1/2 and anti-phospho-p38 MAPK Abs were purchased from New England Biolabs (Beverly, MA). The anti-phospho-ERK1/2 Ab is specific for phosphorylated tyrosine 204 of ERK1/2, and the anti-phospho-p38 Ab is specific for phosphorylated tyrosine 182 of p38 MAPK. Yeast-derived human rTNF was kindly provided by Dr. H. H. Chung, Biotech Research Institute, LG Chem., Daejon, Korea. Human IL-4 was purchased from Genzyme (Cambridge, MA), PMA was purchased from Sigma (St. Louis, MO), and SB203580 and PD98059 were purchased from Calbiochem (La Jolla, CA).

Preparation of sCD40L

Based on the reports describing that the region from glycine 116 to leucine 261 of the extracellular domain of CD40L is self-trimerized (16) and the soluble trimeric CD40L is biologically active (17, 18), the corresponding region was PCR-amplified from Jurkat T cell cDNA library using primers: forward primer: 5′-ATTCTTACGTGGTGAATGCTTGC-3′, reverse primer: 5′-GGAAGCGGCCGCTCAGAGTTTGAGTAAGCCAAAGG-3′, each of which contained a sequence for a restriction site (NheI or NotI). The PCR product was gel purified, phenol extracted, and excised with NheI and NotI. The fragment was cloned into a pCDM7-derived plasmid containing a leader sequence of erythropoietin (19). The resulting expression plasmid was transiently transfected into 293T cells by the CaPO4-DNA precipitation method using N.A.-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid buffer as described elsewhere. At 72 h posttransfection, the supernatant of the transfectant was harvested and analyzed by Western blot for protein expression with the anti-CD40L Ab (C-20). The supernatant was mixed with 0.5% SDS-containing Laemmli buffer without dithiothreitol and reduced at 4°C. The samples were boiled for 3 min. Samples were resolved on 12% SDS-PAGE gel and transferred to nitrocellulose. The lysates were mixed with 20 μl of GST-c-Jun bound to Glutathione Sepharose beads (Pharmacia Biotech, Uppsala, Sweden). Following stimulation, HK cells were lysed in the lysis buffer described in the previous section. The lysates were mixed with 20 μl of GST-c-Jun bound to Glutathione-Sepharose beads. The mixture was rotated at 4°C for 1 h and pelleted by centrifugation at 10,000 rpm for 3 min. The pellets were washed two times with the lysis buffer and once with kinase buffer (20 mM HEPES, pH 7.6, 20 mM MgCl2, 20 mM β-glycerol phosphate, 0.2 mM sodium orthovanadate, 10 mM sodium fluoride, and 0.2 mM DTT) and then resuspended in 50 μl of kinase buffer containing 50 μM ATP and 5 μCi of [γ-32P]ATP (3000 Ci/mmol, Amersham). After a 30-min incubation at 30°C, the reaction was terminated by adding 2× Laemmli sample buffer and boiling for 3 min. Samples were resolved on 12% SDS-PAGE gel and subjected to autoradiography.

RNase protection assay

Confluent cultures of HK or LF-1 cells were left unstimulated or stimulated in 100-mm dishes for 4 h with 1:20 dilution of the 293T supernatant containing sCD40L, 20 ng/ml of TNF-α, or 10 ng/ml PMA. Total RNA was extracted using Tri Reagent (Molecular Research Center, Cincinnati, OH) and 10 μg RNA was analyzed using the Riboquant multiprobe RNase protection assay kit (PharMingen, San Diego, CA), according to the manufacturer’s instructions. In brief, human cytokine/chemokine multi-probe set (hCK-4) was labeled with [α-32P]UTP. Sample RNA was hybridized overnight with the 32P-labeled probes and subjected to RNase digestion. The protected probes were resolved on a 6% denaturing polyacrylamide gel.

Results

Cytokine gene induction by sCD40L and TNF in human FDC line, HK cells

We have chosen sCD40L as an agent for triggering cell surface CD40 on HK cells. The produced sCD40L mainly exists as trimer and binds to CD40. Its biological activity was confirmed by a tonsillar B cell-proliferating activity along with IL-4 (see Materials and Methods and Fig. 1). To confirm that CD40 expressed on HK cells is functional, we analyzed the effect of sCD40L on cytokine gene induction in HK cells. Since CD40-mediated signaling events have been established in human diploid fibroblasts (21, 22), we included this cell type for comparison of sCD40L-activated gene induction profiles with those of HK cells. In parallel, we also compared the effects of TNF on HK cells because TNF belongs to the same superfamily of proteins with CD40L and exhibits similar biological actions in several cell types (21, 23). Total RNA samples were isolated from HK cells and LF-1 cells (human fetal lung...
fibroblast), which were stimulated with 1:20 dilution of the supernatant containing sCD40L or 20 ng/ml TNF for 4 h, and RNase protection assays were performed using human cytokine/chemokine multi-probe template set. As shown in Fig. 2, both sCD40L and TNF increased the level of M-CSF and G-CSF mRNA in HK cells. While TNF strongly enhanced IL-6 mRNA, its expression was not affected by sCD40L. This result was somewhat surprising in view of the fact that CD40 triggering on other cell types such as fibroblasts (21, 22), endothelial cells (24), and monocytes (25) results in IL-6 production. In fact, in agreement with other reports, sCD40L strongly induced IL-6 gene in LF-1 cells with similar efficiency as did TNF, thus excluding the possibility that the dose of sCD40L treated on HK cells was not sufficient to induce IL-6. Fig. 2 also shows that the responses of HK cells to sCD40L or TNF treatment differed from those of LF-1 cells, in that both molecules induced leukemia-inhibitory factor (LIF) mRNA in LF-1 cells, but not in HK cells. HK cells expressed lower levels of M-CSF, G-CSF, and IL-6 mRNAs constitutively, whereas those mRNAs were not detectable in LF-1 cells. This differential effect of CD40 stimulation on cytokine mRNA induction suggests the functionally different roles of CD40 signaling events in different cell types. CD40 signaling in fibroblasts or endothelial cells could influence the extent and the outcome of inflammatory responses by the secretion of inflammatory mediators such as IL-6 and LIF. On the other hand, the capability of HK cells to induce M-CSF and G-CSF mRNA in response to sCD40L suggests that, under physiological conditions, the outcome of CD40 engagement in FDC, which is mediated by CD40L expressed on activated T cells, could result in augmenting their functions during GC reactions.

sCD40L and TNF activate ERK1/2 in HK cells

Since the differential effects of sCD40L and TNF on IL-6 mRNA expression may be due to distinct signaling events by these two molecules, we analyzed the involvement of MAPK family in the activation of HK cells by sCD40L or by TNF. Initially, we determined whether sCD40L or TNF treatments on HK cells were accompanied by the increased tyrosine phosphorylation of ERK1 and ERK2. Confluent cultures of HK cells were exposed to sCD40L or

FIGURE 1. Characterization of recombinant sCD40L. A, sCD40L is glycosylated, and exists mainly as trimer. Supernatants from 293T cells transfected with pCDM-EPOL-sCD40L were incubated with 5 U of PNGase F (left lane) or without enzyme (right lane) at 37°C. Samples were mixed with 0.5% SDS under nonreducing conditions and resolved on a 12% polyacrylamide gel containing 0.5% SDS at 4°C. Proteins were analyzed by Western blotting using anti-CD40L Ab (C-20). B, sCD40L binds to CD40-Fc. The sCD40L-containing supernatants were incubated with Fas-Fc or with CD40-Fc. The bound sCD40L to the respective Fc fusion proteins were precipitated using protein A-Sepharose beads and blotted with anti-CD40L Ab (C-20). C, sCD40L proliferates tonsillar B cells in the presence of IL-4. Purified tonsillar B cells were left untreated (lane 1) or treated with IL-4 (50 U/ml) alone (lane 2), with 1:20 dilution of the supernatant of 293T transfected with sCD40L expression plasmid (lane 3), with IL-4 plus the same dilution of sCD40L-containing supernatant (lane 4), or with IL-4 plus the 1:20 dilution of mock transfected 293T culture medium (lane 5). After pulsing with [3H]thymidine for 16 h, the total incorporated radioactivity was measured as described in Materials and Methods.

FIGURE 2. Cytokine mRNA induction by sCD40L, anti-CD40 mAb (G28-5), or TNF in FDC-line HK cells and in fibroblast LF-1 cells. The confluent cultures of HK cells or LF-1 cells were treated with 1:20 dilution of sCD40L-containing 293T supernatant, with 20 μg/ml of G28-5, or with 20 ng/ml of TNF for 4 h. Total RNA was purified and subjected to RNase protection assay using human cytokine multiprobe hCK4 from PharMingen.
TNF for various intervals. Cell lysates were subjected to Western blot analysis with an aid of phosphotyrosine-specific ERK1/2 Ab. As shown in Fig. 3A, the ERK1/2 Ab identified an increased phosphorylation of 42 and 44 kDa proteins in both sCD40L- and TNF-stimulated HK cells. Increased phosphorylation by sCD40L was evident after 5 min, it reached maximum at 15 min, then decreased to the basal level. TNF showed a delayed but sharper ERK1/2 induction pattern than sCD40L. When HK cells were preincubated with 50 μM PD098059 and subsequently stimulated with sCD40L or with TNF for 15 min. Western blot of the phosphorylated ERK1/2 is shown.

sCD40L induces JNK activity to a very little extent, if at all, but TNF strongly activates it

CD40 engagement preferentially induces JNK activity in most B cells (27–30), where the events leading to the activation of MAPK family induced by CD40 ligation have been primarily characterized. Hence, we investigated whether sCD40L or TNF activates JNK in HK cells. The activity of JNK was measured by the phosphorylation of the substrate, a GST fusion protein containing the N-terminal 96 amino acids of c-Jun. sCD40L appears not to activate JNK as there was a very slight increase in the phosphorylation of GST-c-Jun compared with the basal level, if taken into account. However, TNF caused a strong activation equivalent to about 30-fold increase in JNK activity at 20 min (Fig. 4). The distinct effect of CD40 and TNFR ligation on JNK activation suggests that the signals triggered by these receptors are different although they are structurally homologous and share some associating molecules that interact to the cytoplasmic domain of both receptors.

sCD40L and TNF weakly activate p38 MAPK in HK cells

Sutherland et al. (27) have demonstrated that CD40 ligation in WEHI B lymphoma cell line results in a strong JNK activation without any measurable ERK1/2 activation, differing with the pattern of CD40-mediated activation of MAPK family in HK cells. In addition to JNK activation, WEHI cells respond to CD40 ligation by inducing p38 MAPK activity. Therefore, we then attempted to determine whether sCD40L or TNF activates p38 in HK cells. As shown in Fig. 5A, stimulation of HK cell cultures with either sCD40L or TNF resulted in a transient increase in tyrosine phosphorylation of p38, which showed similar activation kinetics. p38 activation was evident after 5-min treatment of sCD40L or TNF, it reached maximum at 30 min, and then decreased to the basal level. Densitometric scanning of the band intensities showed that the phosphorylation of p38 by TNF was 3.6 times higher than that of sCD40L, indicating that sCD40L is a weaker activator of p38 than TNF, albeit neither is a strong p38 activator when compared with that induced by PMA treatment on HK cells (see Fig. 6B).

To further confirm p38 activation by sCD40L treatment on HK cells, in vitro kinase assay was performed utilizing a GST fusion protein containing the N-terminal 96 amino acids of activating transcription factor 2 (ATF2) as substrate. Although ATF2 is known to serve as substrate for both p38 and JNK (31), given our result that sCD40L did not induce JNK activity, the ATF2 phosphorylation is thought to be governed mostly by the p38 activity induced after stimulating HK cells with sCD40L. In our experiment, therefore, cell lysates were directly used for the assay, instead of immunopurifying p38 from the cell lysates. In addition, SB020358, an inhibitor of p38 MAPK (32), was used to ascertain that the ATF2-phosphorylating activity of HK cells treated with sCD40L was indeed contributed by p38 activation. As shown in Fig. 5B, treating the cells with sCD40L or G28-5 resulted in a modest increase in the phosphorylation of ATF2, and the activity was severely diminished by pretreatment of HK with SB020358. On the other hand, TNF profoundly induced ATF2-phosphorylating activity in HK cells, but the ability of TNF to induce ATF2 phosphorylation was slightly affected by pretreating the cells with SB020358, indicating that the TNF-induced ATF2 phosphorylation is mostly contributed by JNK activity.

Activation of ERK1/2 and p38 by sCD40L is phorbol ester-sensitive PKC-dependent while in the case of TNF, it is PKC independent

To compare early signaling events coupling to ERK1/2 and p38 by sCD40L and by TNF, we examined the effects of PMA-sensitive PKC isotypes. The confluent cultures of HK cells were treated with a high dose (200 ng/ml) of PMA for 24 h in order to deplete PMA-sensitive PKC, and followed by a brief stimulation
of the cells with sCD40L, G28-5, TNF, or PMA for 20 min. Lysates were collected and examined for phosphorylated ERK1/2 or p38. As shown in Fig. 6, PMA pretreatment resulted in a complete inhibition of ERK1/2 and p38 activation by sCD40L or by anti-CD40, but it showed no effect on the cells given TNF stimulation. A brief stimulation of HK cells with a low dose (10 ng/ml) of PMA itself caused both ERK1/2 and p38 activation, but no activation of both kinases in the cells pretreated with the high dose of PMA indicates a complete desensitization of the PKC isoforms. Therefore, PMA-sensitive PKC isoforms are involved in the activation of ERK1/2 and p38 by CD40 ligation on HK cells. This result is different from that observed in B cells in which ERK activation following treatment of anti-CD40 mAb is PMA-sensitive PKC-independent (28, 33), but Ras dependent (34), reflecting distinct characteristics of CD40 signaling in FDC. In contrast, PMA-sensitive PKC isoforms are not involved in the activation of ERK1/2 and p38 by TNF, which is consistent with the result observed in normal human diploid fibroblast cells (35). Activation of ERK by members of the PKC superfamily has been extensively studied by Schönwasser et al. (36), some of which are unaffected by the treatment of phobol ester. One such isoform is PKC-ζ, shown to be activated by TNF (37). Therefore, it is plausible that a TNF-induced signaling pathway in HK cells may involve atypical forms of PKC such as PKC-ζ.

**FIGURE 5.** sCD40L and TNF weakly activate p38 MAPK, and the phosphorylation of ATF2 by sCD40L or by G28-5 is inhibited by pretreating the cells with a p38 inhibitor SB203580. A, HK cells were stimulated with either sCD40L-containing 293T supernatant (1:20 dilution) or with TNF (20 ng/ml) for the indicated times. Lysates were resolved on a 12% SDS-PAGE along with the positive control for phosphorylated p38 supplied by New England Biolabs (indicated as STD (pp38)), and blotted with anti-phospho-p38 Ab. The representative result among three independent experiments is shown. B, HK cells were pretreated with either DMSO or 10 μM SB203580 and subsequently stimulated with sCD40L or with TNF for 15 min. Lysates were mixed with 20 μl of GST-ATF2 bound to GST-Sepharose beads. The mixture was rotated at 4°C for 1 h and pelleted by centrifugation at 10,000 rpm for 3 min. The pelleted beads were washed two times with lysis buffer and once with kinase buffer and then resuspended in 50 μl of kinase buffer containing 50 μM ATP and 10 μCi of [γ-32P]ATP. After 30-min incubation at 30°C, the reaction was terminated by adding 2× Laemmli sample buffer and boiling for 3 min. Samples were resolved on 12% SDS-PAGE and subjected to autoradiography.

**FIGURE 6.** Effects of pretreatment with a high dose of PMA on the activation of ERK1/2 and p38 by sCD40L, anti-CD40 Ab G28-5, or TNF. HK cells were either left untreated or treated for 24 h with 200 ng/ml PMA prior to stimulation for 20 min with 1:20 dilution of sCD40L-containing 293T supernatant, with 20 μg/ml G28-5, with 20 ng/ml of TNF, or with PMA (10 ng/ml). Lysates were then generated, blotted, and probed with anti-phospho-ERK Ab (A) and with anti-phospho-p38 Ab (B). STD (pp38) indicates positive control for phosphorylated p38.

**FIGURE 7.** A and B illustrate that the simultaneous treatment of sCD40L and TNF induced M-CSF and G-CSF mRNA far stronger than those induced by sCD40L or TNF treatment alone, suggesting that different signals induced by the two molecules can converge at distal events in an additive or synergistic manner. This synergism is likely to render FDC more effective in functioning during GC reactions.
is increased by CD40 ligation. The incubation of purified GC B cells with G-CSF results in the rescue of GC B cells from apoptosis (41). Therefore, increased G-CSF mRNA following sCD40L treatment on HK cells suggests that the role of FDC in rescuing spontaneous apoptosis of GC B cells is partly attributed to their capacity to produce G-CSF upon contact with activated T cells via CD40-CD40L interaction.

One intriguing observation we have made in this study is that CD40 stimulation of HK cells by sCD40L or agonistic CD40 mAb (G28-5) did not lead to IL-6 mRNA enhancement, whereas TNF strongly induced it. It has been reported that under pathological conditions, e.g., Castleman's disease, IL-6 mRNA and protein are produced by FDC (42). Recent study has also clearly demonstrated that IL-6 mRNA is produced by the FDC-enriched populations (not by the lymphocytes) within the GC clusters (43). In addition, many cultured FDC lines including HK cells spontaneously produce IL-6, and its level is enhanced after treatment with either IFN-γ or TNF (3, 11). Although spontaneous expression of IL-6 mRNA in HK cells was detected in our RNase protection assay (Figs. 2 and 7), its level was not further enhanced following CD40 stimulation of HK cells. Activation of IL-6 gene in various cell types has been well established (44). Multiple regulatory elements in the IL-6 promoter mediate inducible and tissue-specific transcription regulation by a variety of stimuli. Among them, an NF-κB-binding element is reported to be crucial for IL-6 induction by TNF (45–47) or CD40 stimulation (21). It has been known that M-CSF and G-CSF induction by various stimuli is under control of NF-κB activation (48, 49). Therefore, the inability of HK cells to induce IL-6 gene by sCD40L is not likely due to the defect in the activation of NF-κB, rather it may be due to the differential usage of a set of homo- or heterodimers of proteins belonging to the Rel/NF-κB family whose activation by sCD40L is cell type and target gene dependent. Alternatively, it is known that transcription factors of Fos/Jun, ATF/CREB, NF-IL-6, and Rel/NF-kB families are synergistically operative in inducing maximal IL-6 gene transcription upon stimulation with various agonists (44). Therefore, it is possible that the inability of HK cells to induce IL-6 gene by CD40 stimulation may be due in part to the functional inhibition of the activation pathways for some transcription factors other than NF-κB essential for full IL-6 gene activation.

The fact that sCD40L and TNF induced a common set of cytokine mRNAs (G-CSF and M-CSF induction by HK cells; IL-6, G-CSF, and LIF induction by LF-1 cells) but showed different effects on IL-6 induction by HK cells suggests that they transmit overlapping and/or non-overlapping signals depending on the cell type. This conclusion was supported by the observation that sCD40L and TNF had differential abilities to activate MAPK protein family in HK cells. Both molecules activated ERK1/2 and less pronouncedly p38. While TNF strongly induced JNK activity, sCD40L caused a marginal increase, if at all. Moreover, the requirement for proximal signaling elements leading to ERK1/2 and p38 activation was distinct from each other. sCD40L requires a conventional phosphor ester-sensitive PKC, whereas TNF does not. Whether the differences in PKC requirement of sCD40L and TNF actions could affect IL-6 mRNA induction will be determined in the future experiments.

Berberich et al. (29) reported that cross-linking CD40 by anti-CD40 mAb on Daudi B cell line or human tonsillar B cells preferentially induces JNK rather than ERK. A similar observation was obtained by using human Burkitt’s lymphoma cell line Ramos by Sakata et al. (30). Our observation that sCD40L is a poor activator of JNK in HK cells differs from the strong JNK activation after CD40 ligation on the B cell system. This difference does not seem to be due to subtle variations in agonistic properties of sCD40L.
and anti-CD40 Ab failed to increase JNK activity in HK cells (data not shown); rather, it suggests that CD40-mediated activation pathways of B cells are, at least in part, different from those of FDC. In supporting this notion, a recent study has revealed that monocytes and dendritic cells from patients with CD40L-positive hyper-IgM syndrome due to a defect in CD40 signaling pathway in B cells show normal CD40-mediated cytokine production as well as expression of activation markers CD83, CD86, and CD80, indicating that CD40-mediated signaling in mononuclear/dendritic cells is different from that in B cells (50). Therefore, we speculate that a component of the CD40-JNK pathway may be functionally or physically missing in HK cells, or the pathway is inhibited. TNF-receptor-associated factor 2 (TRAF2), which exhibits the JNK-inducing activity among the known TRAF members and is involved in CD40 and TNFR1 signaling (51), may not play a role in CD40-mediated activation pathways of HK cells. In this respect, it is of interest that Kashiwada et al. (52) have recently reported that TRAF6, another member of the TRAF family, mediates signaling pathway which couples CD40 to ERK activation in a Ras-independent manner. The CD40-mediated ERK activation in HK cells appears to be Ras independent because PKC desensitization by high dose PMA pretreatment blocks its activation. Therefore, it is likely that CD40-induced signal transduction pathways in HK cells involve TRAF6 rather than TRAF2. Similarly, the PMA-sensitive PKC dependency of HK cells to induce ERK activation is different from B cells, in which activation of ERK was reported to be PKC independent (28, 33), but Ras dependent (34).

In contrast to PKC-dependent sCD40L actions in HK cells, TNF did not require PMA-sensitive PKC activity for the activation of ERK and p38, which is in accordance with the results observed in human normal diploid fibroblasts (35). Our finding that simultaneous treatment of sCD40L and TNF on HK cells induces M-CSF and G-CSF mRNAs in a cooperative manner further substantiates the existence of separate pathways in their signaling. The additive or synergistic effect of CD40 and TNF receptor costimulation on HK cells may have functional relevance in regulating the magnitude of the GC reaction by which it makes FDC to be maximally functional by enhancing the quantitative expression of cytokines and/or presumably adhesion molecules, in turn amplifying signals for the three-way communication among FDC, B, and T lymphocytes.

In summary, the data presented here showed that FDC line HK cells functionally activate CD40 molecules, which mediated unique profiles in the induction of cytokine mRNAs. Poor IL-6 inducibility of HK cells by sCD40L suggests a distinct role of CD40 function in FDC, which differs from that of other cell types such as monocytes, endothelial cells, and fibroblasts in which CD40 signal augments the inflammatory responses by secreting such as monocytes, endothelial cells, and fibroblasts in which CD40 function in FDC, which differs from that of other cell types.

Acknowledgments

We thank Dr. Jan Vilcek (New York University Medical Center, New York, NY) for providing comments and criticisms in preparing the manuscript.

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