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Differential expression of cannabinoid CB_2 receptor mRNA in mouse immune cell subpopulations and following B cell stimulation

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Abstract

Cannabinoid CB_2 receptor is reported to be expressed in varying amounts in different human immune subpopulations. To examine the expression pattern of CB_2 in the mouse, immune cell subpopulations were purified and studied by semiquantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR). CB_2 mRNA was most abundant in splenic B cells, followed by macrophages and T cells. Furthermore, CB_2 was expressed in thioglycollate-elicited peritoneal macrophages, but not in resident peritoneal macrophages. In addition to these studies on receptor expression at basal activity, CB_2 mRNA expression was also studied following immune cell activation. Bacterial lipopolysaccharide stimulation downregulated CB_2 mRNA expression in splenocyte cultures in a dose–response manner, while stimulation through cluster of differentiation 40 (CD40) using anti-CD40 antibody upregulated the response and costimulation with interleukin-4 attenuated the anti-CD40 response. These results demonstrate that CB_2 mRNA expression differs among mouse immune subpopulations similar to what is observed in human immune cells. Furthermore, the results suggest that the signaling pathways activated by lipopolysaccharide and anti-CD40 might have different effects on CB_2 mRNA expression. © 2001 Published by Elsevier Science B.V.

Keywords: Cannabinoid CB_2 receptor; Lymphocyte; Lipopolysaccharide; Cluster of differentiation 40 (CD40); Interleukin-4

1. Introduction

The principal psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Gaoni, 1964), exerts its psychoactivity through interaction with a G protein-coupled receptor, cannabinoid receptor 1 (CB_1) in the brain (Matsuda et al., 1990). A second receptor, cannabinoid receptor 2 (CB_2), was cloned from a human promyelocytic leukemia cell line, HL 60 (Munro et al., 1993), and the mouse counterpart was cloned from a mouse splenocyte cDNA library (Shire et al., 1996). Human CB_2 RNA has been found in varying amounts in different human immune subpopulations with the rank order being B cells > natural killer (NK) cells > monocytes > neutrophils > cluster of differentiation 8 (CD8) T-cells > cluster of differentiation 4 (CD4) T cells. In addition to mRNA expression, CB_2 protein expression was detected in tonsils by immunocytochemistry and appeared to be most intense in the B cell enriched areas (Galiegue et al., 1995). Activation of immune cells appears to change cannabinoid receptor expression. For example, we reported that CB_1 receptor message increased following stimulation of the murine macrophage cell line, RAW 264.7 (Klein et al., 1995), and the human T cell line, Jurkat (Daaka et al., 1996). Furthermore, CB_2 mRNA and protein were enhanced in human tonsillar B cell cultures stimulated to proliferation with anticluster of differentiation 40 (CD40) antibody (Carayon et al., 1998). Because of widespread use of the mouse model in immunology research, we felt it important to study the distribution pattern of CB_2 in mouse immune subpopulations and the effects of immune activation on CB_2 expression. We report that, as in humans, CB_2 mRNA is expressed differentially in mouse immune subpopulations and that activating the immune cells changes the expression of CB_2 mRNA.

2. Materials and methods

2.1. Cell preparation and cell culture

Spleens were aseptically removed from 10- to 12-week-old female, BALB/c mice (National Cancer Institute-

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Harlan, Fredricksburg, MD). The mice were maintained in our animal facility that is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Spleen single-cell suspensions were prepared in Hanks balanced salt solution (Sigma, St. Louis, MO) with a Stomacher 80 Lab Blender (Seward, London, UK) and the erythrocytes removed by hypotonic lysis. The splenocytes were washed and resuspended in RPMI 1640 culture medium (Sigma) supplemented with 10% fetal calf serum (HyClone Labs, Logan, UT), penicillin and streptomycin. Splenocytes (2×10^6 cells/ml) were cultured for 4, 6 and 24 h in six-well tissue culture plates (Costar, Corning, NY) in either medium only or stimulated with *Escherichia coli* lipopolysaccharide (10, 1 or 0.1 μg/ml; Sigma), anti-CD40 antibody (100 ng/ml, PharMingen, San Diego, CA) or anti-CD40 plus interleukin-4 (1 ng/ml, PharMingen). In some experiments, B cells were purified from splenocytes using a negative selection kit, Murine B Cell Enrichment Cocktail (Stem Cell Technologies, Vancouver, BC). The purity of B cells was 92%, as determined by flow cytometry. The enriched T cell subpopulation was purified from splenocytes by a two-step procedure. Initially, cells were adhered to plastic for 2 h at 37 °C to remove macrophages. Next, two rounds of adherence to flasks coated with goat anti-mouse immunoglobulin (1 mg/ml; Southern Biotechnology, Birmingham, AL) at a concentration of 45×10^6 cells/25 cm^2 flask were employed to remove B cells. The purity of T cells was 96%, as determined by flow cytometry. Splenic macrophages were obtained by a 2-h adherence followed by an additional 16 h of culture and rinsing to remove dendritic cells.

Resident peritoneal macrophages were collected by peritoneal lavage using 5 ml of phosphate buffered saline (PBS) with 1% of fetal calf serum. Cells were cultured for 2 h, the nonadherent cells discarded, and the adherent cells (macrophages) collected. To obtain thioglycollate-elicited peritoneal macrophages, mice were injected intraperitoneally with 3 ml of thioglycollate broth, and 96 h later, mice were sacrificed and the peritoneal macrophages collected by lavage and purified as above.

2.2. Flow cytometry

To assess immune subpopulation purity, splenocytes (10^6 cells/tube) in PBS were incubated on ice for 30 m with fluorescein-isothiocyanate-labeled anticluster of differentiation 3 (CD3) and phycoerythrin-labeled anticluster of differentiation 19 (CD19; PharMingen) to determine T and B cells, respectively. To assess the extent of cell activation following stimulation, splenocytes were incubated with the above antibodies plus antibodies to the activation marker, cluster of differentiation 69 (CD69; PharMingen). The cells were then washed and fixed in 1% paraformaldehyde and the fluorescent-labeled cells were analyzed with a FACStar flow cytometer (Becton Dickinson, Mountain View, CA).

2.3. RNA isolation

Cells from various cell preparations and cultures were harvested and RNA was extracted with TRI REAGENT™ (Sigma) according to the manufacturer's recommendations. Prior to Reverse Transcription-Polymerase Chain Reaction (RT-PCR), each RNA sample was treated with DNaseI (Boehringer Mannheim, Indianapolis, IN) at room temperature for 1 h.

2.4. Northern blotting

To generate a ^{32}P-labeled CB_2 antisense RNA probe for Northern blotting, the CB_2 gene was subcloned into plasmid pGEM7Zf(−) (Promega, Madison, WI), which contained T7 and SP6 phage RNA polymerase promoters. The pGEM-CB_2 clone was digested with *Hin*dIII and then used in in vitro transcription. A MAXIscript kit (Ambion, Austin, TX) was used, along with T7 phage RNA polymerase, and ^{32}P UTP as the labeling agent. The β-actin antisense RNA probe was produced in the same way using a DNA template provided by Ambion. NorthernMax (Ambion) was used for Northern blotting analysis. In brief, 10 μg of total RNA from each sample was electrophoresed in 1% formaldehyde gel and downward transferred onto a BrightStar-Plus positively charged nylon membrane (Ambion). The membrane was hybridized with CB_2 antisense RNA probe and β-actin antisense RNA probe simultaneously at 65 °C overnight in prehybridization/hybridization (Prehyb/Hyb) solution (Ambion) or 2 h in a rapid Northern blot hybridization (ZIP-Hyb) solution (Ambion). The membrane was then washed and analyzed with a phosphoimager (Molecular Dynamics, Sunnyvale, CA) with ImageQuaNT (Molecular Dynamics) software. The amount of CB_2 expression was normalized as the ratio of counts per minute of CB_2 to β-actin.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

One microgram of RNA was reverse transcribed into cDNA using avian myeloblastosis virus reverse transcriptase (AMV-RT; Promega) and oligo (dT) (Promega) as primer in a 20-μl reaction mixture followed by PCR amplification. For this, 1 μl of RT product and 0.325 μM each of the forward and reverse primers were added in a total of 50 μl PCR reaction mixture using MβP Easy Start PCR Mix-in-a-Tube (Molecular BioProducts, San Diego, CA). The CB_2 and β_2 microglobulin targets were amplified using the following PCR conditions: 94 °C 5 m, 92 °C 40 s, 65 °C 40 s, 75 °C 1 m 30 s, and 75 °C 5 m as the final extension step. The PCR cycles were 38 for CB_2 and 25 for β_2 microglobulin and were observed to be optimal and in the linear portion of the amplification curve (data not shown). CB_2 forward and reverse primers were AACGGTGGCTTGGAGTTCAAC and TAGGTAGCG-

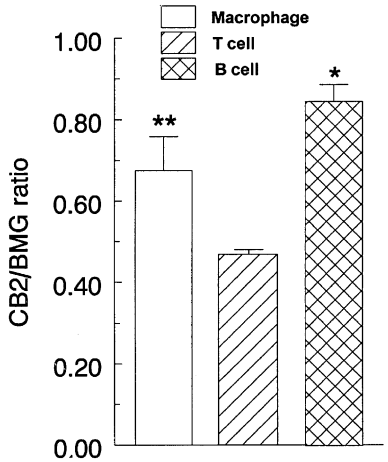

Fig. 1. Cannabinoid receptor 2 (CB_2) mRNA expression in mouse splenic B cells, T cells, and macrophages. Splenic B cells (92% purity), T cells (96% purity) and macrophages were collected, total RNA extracted, and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) performed. PCR products were analyzed on 2% agarose gel followed by densitometry reading. CB_2 mRNA content was normalized with β_2 microglobulin (BMG) and expressed as the ratio of CB_2 /BMG. Data are means ± S.D. of three experiments ($^{*} = P \leq 0.05$, B cells compared to macrophages; $^{**} = P \leq 0.05$, macrophages compared to T cells).

GTCAACAGCGGTTAG, respectively. β_2 microglobulin forward and reverse primers were GGCTCGCTCGGTGACCCTAGTCTTT and TCTGCAGGCGTATGTATCAGTCTCA, respectively. The amplicon sizes were 360 bp (CB_2) and 300 bp (β_2 microglobulin). PCR products were resolved on a 2% agarose gel with ethidium bromide and visualized under UV light. Control RT-PCR reactions were run without reverse transcriptase to verify the samples were negative for contaminating DNA.

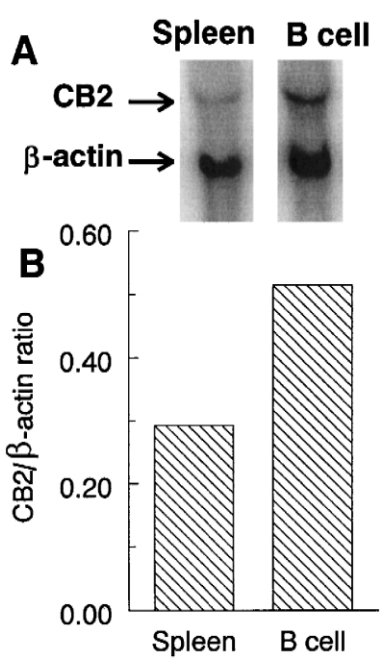

Fig. 2. Northern blotting of CB_2 mRNA expression in splenocytes and splenic B cells. Ten micrograms of total RNA per lane was loaded. Panel A is the phosphoimage of Northern blotting membrane. Panel B is a quantification of A. Panel B is expressed as the count per minute (CPM) ratio of CB_2 / β-actin. Data are representative of five experiments.

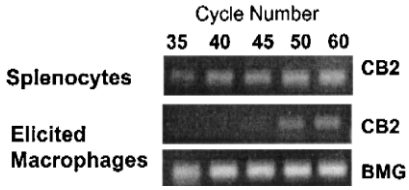

Fig. 3. CB_2 mRNA expression in thioglycollate-elicited peritoneal macrophages. One microgram of total RNA was used in reverse transcription (RT) reaction; subsequently, 1 μl of RT product was used in the PCR reaction, with CB_2 or β_2 microglobulin (BMG) genes being amplified. PCR cycle numbers of 35, 40, 45, 50 and 60 were performed as indicated. CB_2 mRNA expression in splenocytes is shown as a comparison. Data are the representative of three experiments.

2.6. Statistics analysis of data

Data were analyzed by one-way analysis of variance with Dunnett's two-tailed *t* test using SigmaStat (Jandel Scientific, San Rafael, CA).

3. Results

3.1. CB_2 mRNA expression in mouse splenic subpopulations

Total RNA was extracted from splenic B cells, T cells and macrophages, and RT-PCR was performed. Densitometry readings showed that the expression of CB_2 mRNA in splenic subpopulations was in the rank order of B cells > macrophages > T cells (Fig. 1). Northern blotting analysis of the samples was also run to confirm these results. A 4-kb CB_2 and a 2.1-kb β-actin band (Fig. 2A) were observed in splenic RNA. The finding of this size transcript in mouse splenocytes corresponds to previous reports (Condie et al., 1996; Schatz et al., 1997). Furthermore, densitometry analysis showed that expression of CB_2 was relatively higher in B cells with the amount almost double that in unfractionated splenocytes (0.52 ratio vs. 0.29 ratio) (Fig. 2B).

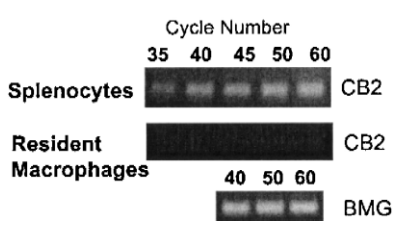

Fig. 4. CB_2 mRNA not detected in resident peritoneal macrophages. RT-PCR was carried out on total RNA derived from resident peritoneal macrophages as described in Fig. 3. The PCR cycle numbers for CB_2 were 35, 40, 45, 50, and 60, and for β_2 microglobulin (BMG) were 40, 50 and 60. CB_2 mRNA expression in splenocytes is shown as a comparison. Data are the representative of three experiments.

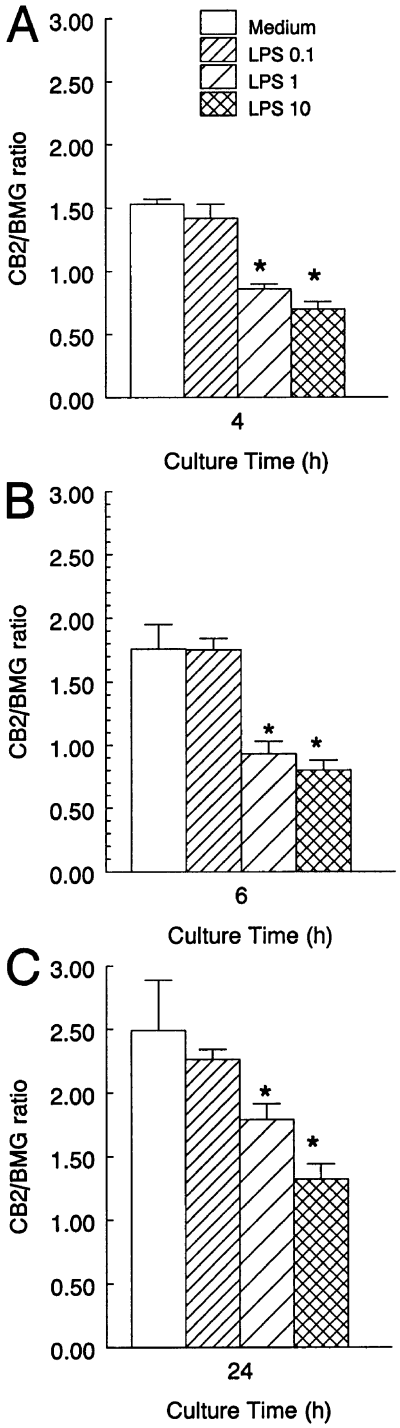

Fig. 5. CB_2 mRNA expression in cultured splenocytes stimulated with bacterial lipopolysaccharide (LPS). Splenocytes were cultured with/without lipopolysaccharide 10, 1, or 0.1 μg/ml for 4 (panel A), 6 (panel B), and 24 (panel C) h and analyzed for CB_2 mRNA expression by RT-PCR (see materials and methods). PCR cycle number was 38 for CB_2, 25 for β_2 microglobulin (BMG). Densitometry readings of RT-PCR results were expressed as the ratio of CB_2 /BMG. Data are the means ± S.D. of three experiments and significant differences were observed ($^* = P \leq 0.05$) when the treated groups were compared to the groups cultured in medium only.

3.2. CB_2 mRNA expression in resident and thioglycollate-elicited peritoneal macrophages

We extended our analysis of CB_2 mRNA expression to peritoneal macrophages. Resident and thioglycollate-elicited cells were harvested as described in Materials and methods. Total RNA was extracted and RT-PCR was performed. Fig. 3 shows that CB_2 mRNA was less abundant in elicited macrophages than in splenocytes. Amplicon signal was evident at 35 cycles in RNA from splenocytes, but was not seen in macrophages until 50 cycles (Fig. 3). Furthermore, amplicon signal was detected in macrophage mRNA at 35 cycles if the input amount of RT product was increased to 5 μl (data not shown). Regarding detection in resident macrophages, RT-PCR amplification failed to produce detectable amplicons even at 60 cycles (Fig. 4), suggesting that, unlike elicited cells, resident cells do not express CB_2 mRNA.

3.3. CB_2 mRNA expression following stimulation of splenocytes with lipopolysaccharide

Very few studies have addressed the issue of CB_2 gene modulation following immune cell activation (Carayon et al., 1998). To investigate the modulation of CB_2 mRNA expression in mouse splenocyte cultures, we initially chose the B cell mitogen, lipopolysaccharide. Splenocytes were

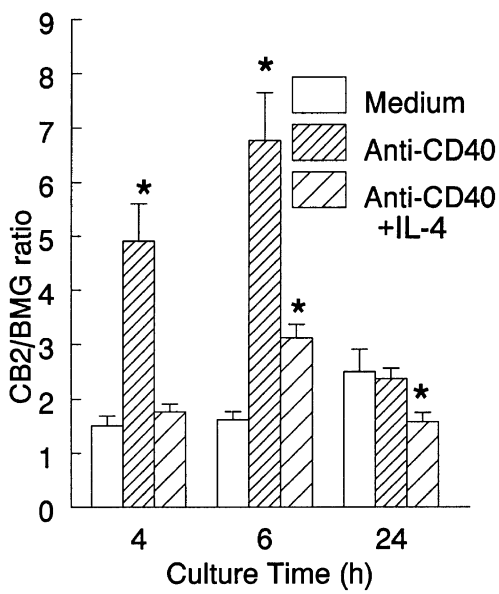

Fig. 6. CB_2 mRNA expression in cultured splenocytes stimulated with anti-CD40 or anti-CD40 plus interleukin-4 (IL-4). Splenocytes were cultured with anti-CD40 (100 ng/ml) with/without interleukin-4 (1 ng/ml) for 4, 6 and 24 h and analyzed for CB_2 mRNA expression by RT-PCR (see Materials and Methods). The PCR cycle number was 38 for CB_2 and 25 for β_2 microglobulin (BMG). Densitometry readings of RT-PCR results are expressed as the ratio of CB2/BMG. Data are means ± S.D. of three experiments and significant differences were observed ($^* = P \leq 0.05$) when the treated groups were compared to the groups cultured in medium only.

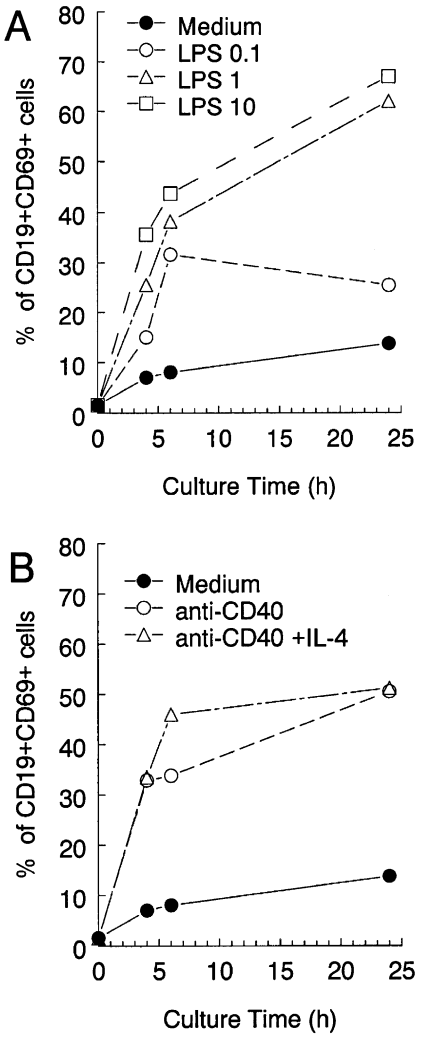

Fig. 7. Mitogen stimulation increased early activation marker, CD69, expression on mouse B cells. Splenocytes were cultured with lipopolysaccharide (LPS) (panel A) or anti-CD40 (100 ng/ml) with/without interleukin-4 (IL-4; 1 ng/ml) (panel B) for 0, 4, 6, and 24 h. Cells were harvested and stained for CD19 (B cells) and CD69 for flow cytometry analysis. Panel A, medium (●), lipopolysaccharide 0.1 (○), lipopolysaccharide 1 (△), and lipopolysaccharide 10 (□) μg/ml. Panel B, medium (●), anti-CD40 (○), and anti-CD40 + interleukin-4 (△). Results are representative of three experiments.

cultured with or without lipopolysaccharide (10, 1 or 0.1 μg/ml) for 4, 6 and 24 h, and CB_2 and β_2 microglobulin mRNA expression analyzed by RT-PCR and densitometry (Fig. 5). Interestingly, incubation of splenocytes for up to 24 h increased the expression of CB_2 relative to β_2 microglobulin, as shown in the medium group in Fig. 5. Lipopolysaccharide treatment, however, dose dependently reduced the expression of CB_2 at 4 h (Fig. 5A), 6 h (Fig. 5B) and 24 h (Fig. 5C).

3.4. CB_2 mRNA expression following stimulation of splenocytes with anti-CD40 or anti-CD40 plus interleukin-4

B cell gene activity and cellular functions can be stimulated by agents other than lipopolysaccharide. For example, CD40 is a B cell receptor recognized as playing a crucial role in the regulation of immune responses (van Kooten and Banchereau, 1997) and anti-CD40 antibodies have been reported to induce mouse B cell proliferation through a pathway distinct from lipopolysaccharide (Nomura et al., 1995). Interleukin-4 has also been found to modulate B cell function and in costimulation with anti-CD40 appears to drive antibody class switching to immunoglobulin G1 (IgG1) and immunoglobulin E (IgE) production (Kaneko et al., 1996). Therefore, we thought it of interest to examine CB_2 mRNA expression in splenocytes treated with agents other than lipopolysaccharide and that stimulate B cells through different signaling pathways. Splenocytes were cultured with anti-CD40 antibody (100 ng/ml) with or without interleukin-4 (1 ng/ml) for 4, 6 and 24 h. RT-PCR was performed on total RNA derived from each cell culture group and mRNA expression was analyzed by densitometry and expressed as the ratio of

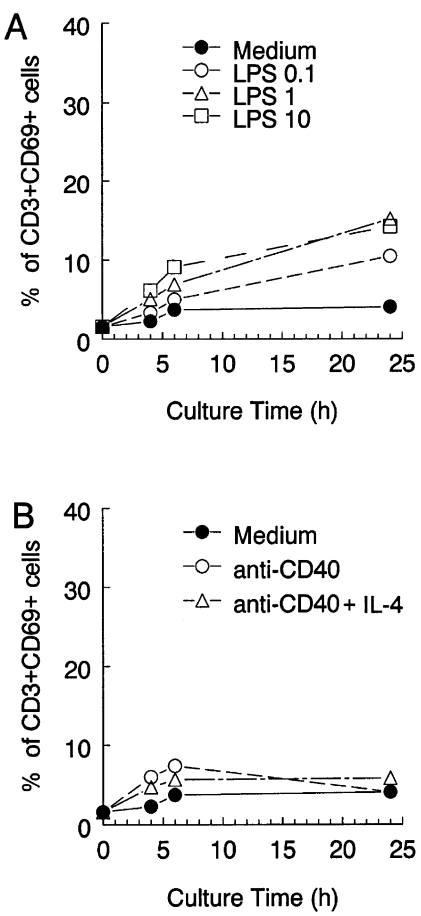

Fig. 8. Mitogen stimulation does not increase early activation marker, CD69, expression on mouse T cells. Splenocytes were cultured with lipopolysaccharide (LPS) (panel A) or anti-CD40 (100 ng/ml) with/without interleukin-4 (IL-4; 1 ng/ml) (panel B) for 0, 4, 6, and 24 h. Cells were harvested and stained for CD3 (T cells) and CD69 for flow cytometry analysis. Panel A, medium (●), lipopolysaccharide 0.1 (○), lipopolysaccharide 1 (△), and lipopolysaccharide 10 μg/ml (□). Panel B, medium (●), anti-CD40 (○), and anti-CD40 + interleukin-4 (△). Results are representative of three experiments.

CB_2 to β_2 microglobulin (Fig. 6). Data indicated that CB_2 mRNA expression was upregulated following stimulation with anti-CD40 antibodies at 4 and 6 h, and declined at 24 h. However, costimulation with interleukin-4 attenuated the anti-CD40 increase (Fig. 6).

3.5. B cells rather than T cells are activated by lipopolysaccharide, anti-CD40 or anti-CD40 plus interleukin-4

Because we used unfractionated splenocytes in some of our studies, we wanted to verify that lipopolysaccharide and anti-CD40 treatments predominantly stimulated the splenic B cell subpopulation. Duplicate cell cultures were set up and at various times were stained for CD3 (T cells), CD19 (B cells) and CD69, an early activation marker on lymphocytes (Ziegler et al., 1994). The results showed that the activated B cells, identified as $CD19^+$ $CD69^+$, increased substantially with time through 24 h following treatment with lipopolysaccharide 10 or 1 $\mu g/ml$ (Fig. 7A). Treatment with only $0.1 \mu g/ml$ lipopolysaccharide, however, resulted in an increase through only 6 h following stimulation followed by a plateau in the percentage of $CD69^+$ B cells. In both anti-CD40 and anti-CD40 plus interleukin-4 stimulated groups, the percentage of $CD19^+$ $CD69^+$ cells increased through the 24-h incubation period (Fig. 7B). Regarding T cell stimulation, lipopolysaccharide had a very slight effect on CD69 expression (Fig. 8A), while CD40 $\pm$ interleukin-4 had no effect (Fig. 8B). This suggests that, as expected, the B cells rather than T cells were primarily activated under these conditions.

4. Discussion

CB_2 was cloned from a mouse splenocyte cDNA library as a 3.7-kb transcript (Shire et al., 1996). Subsequently, a 4.0-kb transcript was demonstrated in mouse splenocytes (Condie et al., 1996; Schatz et al., 1997) and we have observed in the current study a similar-sized transcript in our splenocyte preparations. Regarding CB_2 expression in lymphocyte subpopulations, human B cells, isolated from peripheral blood mononuclear cells (PBMCs), were found to have the highest level of CB_2 mRNA (Galiegue et al., 1995), and we have also found that mouse splenic B cells have more than T cells. From this, it appears that the relatively high level of CB_2 expression in B cells may be a general occurrence in that it has now been observed in both human PBMCs and mouse splenocytes.

Human CB_2 was originally cloned from a promyelocytic leukemia cell line, HL60. A similar transcript was identified in rat spleen and was reported to be expressed in macrophages more abundantly than in T cells (Munro et al., 1993). Our results show that CB_2 is also expressed in mouse splenic macrophages and possibly at higher levels than in T cells. Interestingly, however, not all mouse macrophage subpopulations were positive for CB_2 expression. For example, resident peritoneal macrophages were negative for message expression, while thioglycollate-elicited cells expressed CB_2 transcripts. We have also detected the expression of CB_2 in a mouse alveolar macrophage cell line, designated MH-S (data not shown). Because resident peritoneal cells represent a relatively quiescent population (Rezzani et al., 1999), our findings demonstrate diversity in CB_2 expression among macrophages and suggest preferential expression in inflammatory macrophages (thioglycollate-elicited macrophages) and transformed macrophages cell lines (simian virus 40 transformed, MH-S) rather than quiescent, resident cells.

Previous evidence suggests that activation of immune cells modulates the expression of cannabinoid receptors (Klein et al., 1995; Daaka et al., 1996). Furthermore, it has been reported that human CB_2 mRNA and protein expression of tonsillar B cells were increased within 24 h following stimulation with anti-CD40 antibody (Carayon et al., 1998). Interestingly, however, in that study, CB_2 mRNA sharply decreased to baseline 24 h later (i.e., 48 h after stimulation). In the current study, we found in mouse cells that CB_2 mRNA was increased in a transient fashion following stimulation with anti-CD40. It increased at 4 and 6 h, and decreased by 24 h after stimulation. Thus, although the kinetics of the response differed, the overall trend was the same.

Interleukin-4 has been found to play a crucial role in costimulation with anti-CD40 in the differentiation and class-switching to IgG1 and IgE in B cells (Kaneko et al., 1996). Therefore, we tested interleukin-4 in conjunction with anti-CD40 in our experiments. Cotreatment with interleukin-4 attenuated the anti-CD40-induced increase in CB_2 mRNA, suggesting that the signals generated by interleukin-4 had an inhibitory effect on CB_2 gene expression. Interleukin-4 has been reported to suppress in B cells other gene targets, such as interleukin-12, that are increased by anti-CD40 (Schultze et al., 1999).

In addition to anti-CD40, we also studied the effect of lipopolysaccharide stimulation on CB_2 mRNA expression. We observed that this stimulator induced a decrease rather than an increase in CB_2 expression. Furthermore, from flow cytometry studies, we observed that anti-CD40, anti-CD40 plus interleukin-4 and lipopolysaccharide stimulation increased the expression of the early activation marker, CD69, on B cells rather than T cells. These data demonstrate that, although both anti-CD40 and lipopolysaccharide activated the B cells, these stimulators have opposite effects on CB_2 mRNA expression with CD40 causing an increase, while lipopolysaccharide causing a decrease. This suggests a different regulation of the response by these stimulators. Regarding the mechanisms of CB_2 mRNA modulation, the receptors have been observed to be downregulated in human tonsillar B cells during differentiation to mature germinal center cells (Carayon et al., 1998). Furthermore, in response to anti-CD40, CB_2 message was increased, while in response to anti-CD40 plus interleukin-

4, and lipopolysaccharide, the message was decreased, suggesting that receptor expression is linked to different activation pathways stimulated by these agents. This is supported by observations that anti-CD40, lipopolysaccharide and interleukin-4 cause a variety of effects on B cell differentiation (Deenick et al., 1999; Maliszewski et al., 1993) and that lipopolysaccharide has been observed to downregulate other seven-transmembrane receptors in mice (Zhou et al., 1999). It is also reported that gene activation pathways differ following stimulation with either lipopolysaccharide or anti-CD40. For example, lipopolysaccharide stimulates B cells via a phosphatidylinositol 3-kinase-dependent signaling pathway (Venkataraman et al., 1999). On the other hand, anti-CD40 stimulates through pathways including NF-κB, c-*Jun N*-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK), protein-tyrosine kinases and phosphatases (Pullen et al., 1999). It is possible, therefore, that the lipopolysaccharide signaling pathway is linked to a downregulation of CB_2 gene expression, while the CD40 pathway is linked to an upregulation.

In summary, CB_2 mRNA is differentially expressed on mouse immune subpopulations and, in addition, different B cell mitogens induce the modulation of the receptor to different degrees. Additional studies are needed to determine the precise regulatory effects of lipopolysaccharide and anti-CD40 on CB_2 expression and the role of CB_2 expression in B cell proliferation and differentiation.

Acknowledgements

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