

Differential expression of cannabinoid CB₂ receptor mRNA in mouse immune cell subpopulations and following B cell stimulation

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Received 29 May 2001; accepted 1 June 2001

Abstract

Cannabinoid CB₂ receptor is reported to be expressed in varying amounts in different human immune subpopulations. To examine the expression pattern of CB₂ in the mouse, immune cell subpopulations were purified and studied by semiquantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR). CB₂ mRNA was most abundant in splenic B cells, followed by macrophages and T cells. Furthermore, CB₂ was expressed in thioglycollate-elicited peritoneal macrophages, but not in resident peritoneal macrophages. In addition to these studies on receptor expression at basal activity, CB₂ mRNA expression was also studied following immune cell activation. Bacterial lipopolysaccharide stimulation downregulated CB₂ 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 CB₂ 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 CB₂ mRNA expression. © 2001 Published by Elsevier Science B.V.

Keywords: Cannabinoid CB₂ 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 (CB₁) in the brain (Matsuda et al., 1990). A second receptor, cannabinoid receptor 2 (CB₂), 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 CB₂ 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, CB₂ 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 im-

mune cells appears to change cannabinoid receptor expression. For example, we reported that CB₁ 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, CB₂ 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 CB₂ in mouse immune subpopulations and the effects of immune activation on CB₂ expression. We report that, as in humans, CB₂ mRNA is expressed differentially in mouse immune subpopulations and that activating the immune cells changes the expression of CB₂ 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 $\mu\text{g}/\text{ml}$; 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 cm² 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 min 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 ³²P-labeled CB₂ antisense RNA probe for Northern blotting, the CB₂ gene was subcloned into plasmid pGEM7Zf(–) (Promega, Madison, WI), which contained T7 and SP6 phage RNA polymerase promoters. The pGEM-CB₂ clone was digested with *Hind*III and then used in *in vitro* transcription. A MAXIscript kit (Ambion, Austin, TX) was used, along with T7 phage RNA polymerase, and ³²P 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 CB₂ 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 phosphorimager (Molecular Dynamics, Sunnyvale, CA) with ImageQuaNT (Molecular Dynamics) software. The amount of CB₂ expression was normalized as the ratio of counts per minute of CB₂ 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 M β P Easy Start PCR Mix-in-a-Tube (Molecular BioProducts, San Diego, CA). The CB₂ and β_2 microglobulin targets were amplified using the following PCR conditions: 94 °C 5 min, 92 °C 40 s, 65 °C 40 s, 75 °C 1 min 30 s, and 75 °C 5 min as the final extension step. The PCR cycles were 38 for CB₂ and 25 for β_2 microglobulin and were observed to be optimal and in the linear portion of the amplification curve (data not shown). CB₂ forward and reverse primers were AACGGTGGCTTGGAGTTCAAC and TAGGTAGCG-

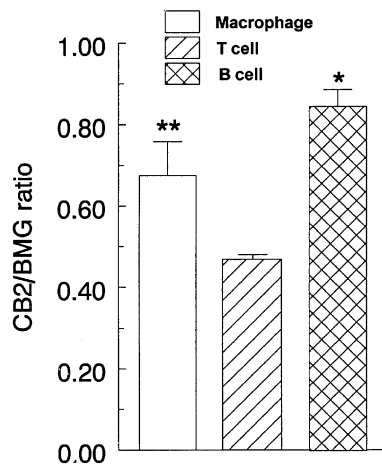


Fig. 1. Cannabinoid receptor 2 (CB₂) mRNA expression in mouse splenic B cells, T cells, and macrophages. Splenic B cells (92% purity), T cells (96% purity) and macrophages were collected, total RNA extracted, and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) performed. PCR products were analyzed on 2% agarose gel followed by densitometry reading. CB₂ mRNA content was normalized with β_2 microglobulin (BMG) and expressed as the ratio of CB₂/BMG. Data are means $\pm$ 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 GGCTCGCTCGGT-GACCCTAGTCTTT and TCTGCAGGCGTATG-TATCAGTCTCA, respectively. The amplicon sizes were 360 bp (CB₂) and 300 bp (β_2 microglobulin). PCR products were resolved on a 2% agarose gel with ethidium

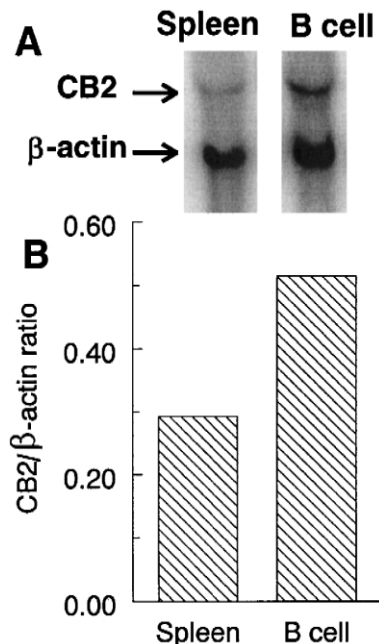


Fig. 2. Northern blotting of CB₂ mRNA expression in splenocytes and splenic B cells. Ten micrograms of total RNA per lane was loaded. Panel A is the phosphorimage of Northern blotting membrane. Panel B is a quantification of A. Panel B is expressed as the count per minute (CPM) ratio of CB₂/ β -actin. Data are representative of five experiments.

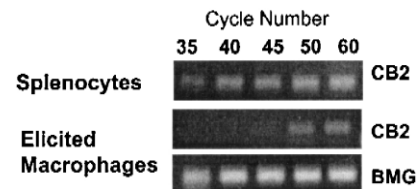


Fig. 3. CB₂ mRNA expression in thioglycollate-elicited peritoneal macrophages. One microgram of total RNA was used in reverse transcription (RT) reaction; subsequently, 1 μ l of RT product was used in the PCR reaction, with CB₂ or β_2 microglobulin (BMG) genes being amplified. PCR cycle numbers of 35, 40, 45, 50 and 60 were performed as indicated. CB₂ mRNA expression in splenocytes is shown as a comparison. Data are the representative of three experiments.

bromide and visualized under UV light. Control RT-PCR reactions were run without reverse transcriptase to verify the samples were negative for contaminating DNA.

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. CB₂ 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 CB₂ 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 CB₂ 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 CB₂ was relatively higher in B cells with the amount almost double that in unfractionated splenocytes (0.52 ratio vs. 0.29 ratio) (Fig. 2B).

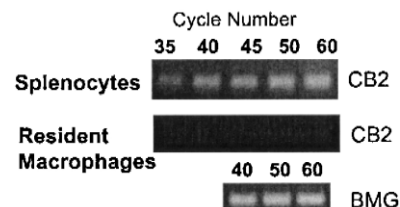


Fig. 4. CB₂ mRNA not detected in resident peritoneal macrophages. RT-PCR was carried out on total RNA derived from resident peritoneal macrophages as described in Fig. 3. The PCR cycle numbers for CB₂ were 35, 40, 45, 50, and 60, and for β_2 microglobulin (BMG) were 40, 50 and 60. CB₂ mRNA expression in splenocytes is shown as a comparison. Data are the representative of three experiments.

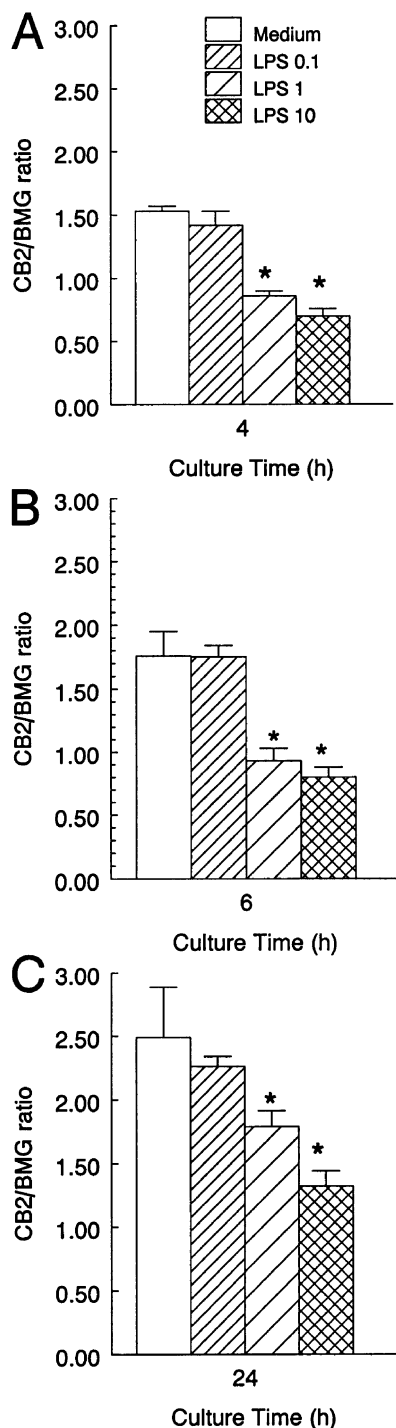


Fig. 5. CB_2 mRNA expression in cultured splenocytes stimulated with bacterial lipopolysaccharide (LPS). Splenocytes were cultured with/without lipopolysaccharide 10, 1, or 0.1 $\mu\text{g}/\text{ml}$ for 4 (panel A), 6 (panel B), and 24 (panel C) h and analyzed for CB_2 mRNA expression by RT-PCR (see 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 $\pm$ 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

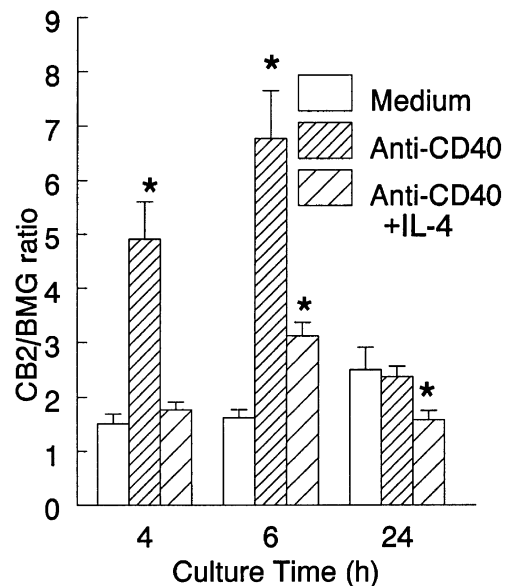


Fig. 6. CB_2 mRNA expression in cultured splenocytes stimulated with anti-CD40 or anti-CD40 plus interleukin-4 (IL-4). Splenocytes were cultured with anti-CD40 (100 ng/ml) with/without interleukin-4 (1 ng/ml) for 4, 6 and 24 h and analyzed for CB_2 mRNA expression by RT-PCR (see Materials and Methods). 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 CB_2 /BMG. Data are means $\pm$ 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.

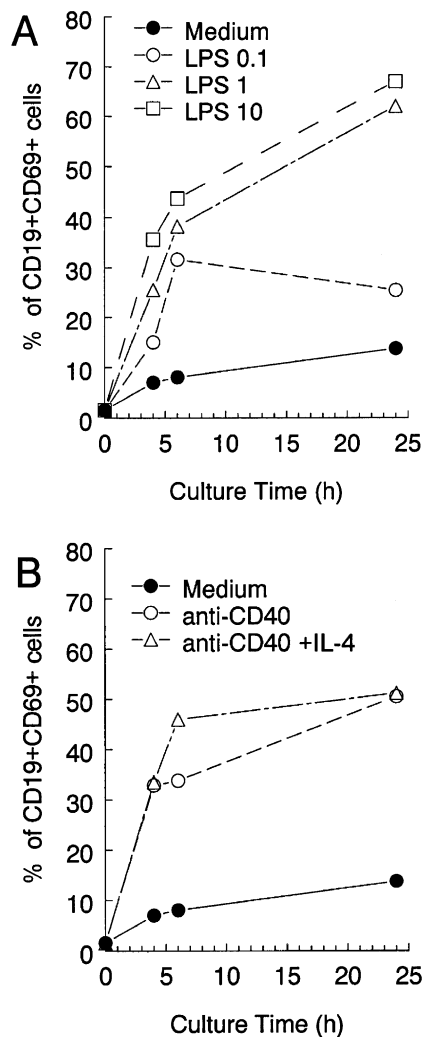


Fig. 7. Mitogen stimulation increased early activation marker, CD69, expression on mouse B cells. Splenocytes were cultured with lipopolysaccharide (LPS) (panel A) or anti-CD40 (100 ng/ml) with/without interleukin-4 (IL-4; 1 ng/ml) (panel B) for 0, 4, 6, and 24 h. Cells were harvested and stained for 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 exam-

ple, 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

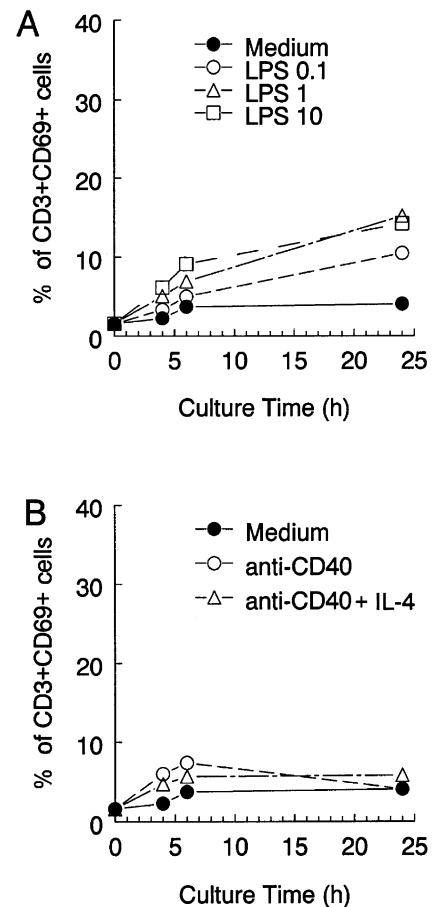


Fig. 8. Mitogen stimulation does not increase early activation marker, CD69, expression on mouse T cells. Splenocytes were cultured with lipopolysaccharide (LPS) (panel A) or anti-CD40 (100 ng/ml) with/without interleukin-4 (IL-4; 1 ng/ml) (panel B) for 0, 4, 6, and 24 h. Cells were harvested and stained 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.

CB₂ to β_2 microglobulin (Fig. 6). Data indicated that CB₂ 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 $\mu\text{g}/\text{ml}$ (Fig. 7A). Treatment with only 0.1 $\mu\text{g}/\text{ml}$ 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

CB₂ 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 CB₂ expression in lymphocyte subpopulations, human B cells, isolated from peripheral blood mononuclear cells (PBMCs), were found to have the highest level of CB₂ mRNA (Galiege 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 CB₂ expression in B cells may be a general occurrence in that it has now been observed in both human PBMCs and mouse splenocytes.

Human CB₂ 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 CB₂ 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 CB₂ expres-

sion. For example, resident peritoneal macrophages were negative for message expression, while thioglycollate-elicited cells expressed CB₂ transcripts. We have also detected the expression of CB₂ 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 CB₂ 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 CB₂ 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, CB₂ mRNA sharply decreased to baseline 24 h later (i.e., 48 h after stimulation). In the current study, we found in mouse cells that CB₂ 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 CB₂ mRNA, suggesting that the signals generated by interleukin-4 had an inhibitory effect on CB₂ 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 CB₂ mRNA expression. We observed that this stimulator induced a decrease rather than an increase in CB₂ 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 CB₂ 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 CB₂ mRNA modulation, the receptors have been observed to be down-regulated in human tonsillar B cells during differentiation to mature germinal center cells (Carayon et al., 1998). Furthermore, in response to anti-CD40, CB₂ 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- κ B, 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 CB₂ gene expression, while the CD40 pathway is linked to an upregulation.

In summary, CB₂ 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 CB₂ expression and the role of CB₂ expression in B cell proliferation and differentiation.

Acknowledgements

This work was supported by grants from the National Institute of Drug Abuse DA10683 and DA07245.

References

- Carayon, P., Marchand, J., Dussossoy, D., Derocq, J.M., Jbilo, O., Bord, A., Bouaboula, M., Galiegue, S., Mondiere, P., Penarier, G., Fur, G.L., Defrance, T., Casellas, P., 1998. Modulation and functional involvement of CB₂ peripheral cannabinoid receptors during B-cell differentiation. *Blood* 92, 3605–3615.
- Condie, R., Herring, A., Koh, W.S., Lee, M., Kaminski, N.E., 1996. Cannabinoid inhibition of adenylyate cyclase-mediated signal transduction and interleukin 2 (IL-2) expression in the murine T-cell line, EL4.IL-2. *J. Biol. Chem.* 271, 13175–13183.
- Daaka, Y., Friedman, H., Klein, T.W., 1996. Cannabinoid receptor proteins are increased in Jurkat, human T-cell line after mitogen activation. *J. Pharmacol. Exp. Ther.* 276, 776–783.
- Deenick, E.K., Hasbold, J., Hodgkin, P.D., 1999. Switching to IgG3, IgG2b, and IgA is division linked and independent, revealing a stochastic framework for describing differentiation. *J. Immunol.* 163, 4707–4714.
- Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., Casellas, P., 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232, 54–61.
- Gaoni, Y.A.M.R., 1964. Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86, 1646–1647.
- Kaneko, Y., Hirose, S., Abe, M., Yagita, H., Okumura, K., Shirai, T., 1996. CD40-mediated stimulation of B1 and B2 cells: implication in autoantibody production in murine lupus. *Eur. J. Immunol.* 26, 3061–3065.
- Klein, T.W., Newton, C., Zhu, W., Daaka, Y., Friedman, H., 1995. Delta 9-tetrahydrocannabinol, cytokines, and immunity to *Legionella pneumophila*. *Proc. Soc. Exp. Biol. Med.* 209, 205–212.
- Maliszewski, C.R., Grabstein, K., Fanslow, W.C., Armitage, R., Spriggs, M.K., Sato, T.A., 1993. Recombinant CD40 ligand stimulation of murine B cell growth and differentiation: cooperative effects of cytokines. *Eur. J. Immunol.* 23, 1044–1049.
- Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. [see comments] *Nature* 346, 561–564.
- Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. [see comments] *Nature* 365, 61–65.
- Nomura, J., Inui, S., Yamasaki, T., Kataoka, S., Maeda, K., Nakanishi, K., Sakaguchi, N., 1995. Anti-CD40 monoclonal antibody induces the proliferation of murine B cells as a B-cell mitogen through a distinct pathway from receptors for antigens or lipopolysaccharide. *Immunol. Lett.* 45, 195–203.
- Pullen, S.S., Dang, T.T., Crute, J.J., Kehry, M.R., 1999. CD40 signaling through tumor necrosis factor receptor-associated factors (TRAFs). Binding site specificity and activation of downstream pathways by distinct TRAFs. *J. Biol. Chem.* 274, 14246–14254.
- Rezzani, R., Rodella, L., Zauli, G., Caimi, L., Vitale, M., 1999. Mouse peritoneal cells as a reservoir of late dendritic cell progenitors. *Br. J. Haematol.* 104, 111–118.
- Schatz, A.R., Lee, M., Condie, R.B., Pulaski, J.T., Kaminski, N.E., 1997. Cannabinoid receptors CB₁ and CB₂: a characterization of expression and adenylyate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* 142, 278–287.
- Schultze, J.L., Michalak, S., Lowne, J., Wong, A., Gilleece, M.H., Gribben, J.G., Nadler, L.M., 1999. Human non-germinal center B cell interleukin (IL)-12 production is primarily regulated by T cell signals CD40 ligand, interferon gamma, and IL-10: role of B cells in the maintenance of T cell responses. *J. Exp. Med.* 189, 1–12.
- Shire, D., Calandra, B., Rinaldi-Carmona, M., Oustric, D., Pessegue, B., Bonnin-Cabanne, O., Le Fur, G., Caput, D., Ferrara, P., 1996. Molecular cloning, expression and function of the murine CB₂ peripheral cannabinoid receptor. *Biochim. Biophys. Acta* 1307, 132–136.
- van Kooten, C., Banchereau, J., 1997. Functions of CD40 on B cells, dendritic cells and other cells. *Curr. Opin. Immunol.* 9, 330–337.
- Venkataraman, C., Shankar, G., Sen, G., Bondada, S., 1999. Bacterial lipopolysaccharide induced B cell activation is mediated via a phosphatidylinositol 3-kinase dependent signaling pathway. *Immunol. Lett.* 69, 233–238.
- Zhou, Y., Yang, Y., Warr, G., Bravo, R., 1999. LPS down-regulates the expression of chemokine receptor CCR2 in mice and abolishes macrophage infiltration in acute inflammation. *J. Leukocyte Biol.* 65, 265–269.
- Ziegler, S.F., Ramsdell, F., Alderson, M.R., 1994. The activation antigen CD69. *Stem Cells* 12, 456–465.