Proteomics Final Project: Option 1 Kaitlyn Terrell

Protein: CCL2 or MCP-1

Alternative Names: C-C Motif Chemokine 2; Monocyte Chemoattractant Protein-1; SCYA2

Function: Involved in inflammation and immune response, also found in retina.

Summary of Findings: (6 databases and 4 publications used to support validation)

Because this protein is involved in inflammation and immune response, in the retina when experiencing infection or disease, this immune response may also cause degradation of the retina. Through the use of multiple databases and supporting publications, it is plausible that CCL2(MCP-1) may be involved in direct or indirect interaction with Rhodopsin in the retina. Starting out with the UniProt Database, we get a general picture of what the CCL2 protein's basic biological and molecular functions. Involved in chemotaxis and immune response, this cytokine can be found on a subcellular level secreted into extracellular space. The Human Protein Atlas database shows us another view of the subcellular level of CCL2 expression, where secretion outside of the cell is noted as a prediction, it primarily labels the Golgi apparatus and Vesicles as this protein's place of expression, supported by some lovely fluorescent cell localization. This database also provides us with a better understanding of where in the body this protein is primarily expressed. While dominant in the spinal cord, our area of interest for today is the retina. We can see in adjacent plots of RNA expression in the human brain and the retina is a location for expression. Additionally, in tissue expression we can see that CCL2 is also expressed in the retina. Single cell type was also analyzed, and in the eye the dominant cell type for CCL2 expression was Muller glia cells. The STRING database allows us to search for our protein of interest, CCL2 and Rhodopsin at the same time to see if they are in the same interactome network. When we zoom out to encompass multiple interactions, we can see that while the two proteins are not directly interacting with one another, they do have known interactions with similar proteins and are interwoven within the same network. To get a more comprehensive view of the known interactions with CCL2, I used the NextProt database to get the PathwayCommons ID for this protein which shows us a different kind of interactome. This is less intuitive than STRING, but allows us to see a wider range of known direct interactions with our protein of interest, again excluding rhodopsin. Out of curiosity, I decided to search KEGG for pathways including CCL2 and rhodopsin or other interesting proteins. I ended up finding several for CCL2, and two or more included direct connections to NF-kappaB. The pathways are not in the retina, but the part of the pathway that connects the two proteins appears to be the NF-kappaB signaling pathway. Out of all of the collected data here, I think that the most convincing validation for further study is the presence of CCL2 expression in the retina in humans, as well as the findings from the publication described below analyzing the MCP-1/CCR2 pathway (doi: 10.1016/j.exer.2012.08.013).

Most Significant Publication: https://doi.org/10.1016/j.exer.2012.08.013

This study done in rd10 mice used RT-PCR to analyze the expression of MCP-1 (CCL2), RANTES, STR-1, and TNF-alpha in the retinas of wild type, rd10, and ccr2 $^{-/-}$ rd10 (ccr2 mutant) mice. They found that the ccr2 $^{-/-}$ rd10 mutants had higher Rhodopsin gene expression than the rd10 (ccr2 $^{+/+}$ rd10) mice, and observed better retinal function preservation. This finding suggests photoreceptor survival. They concluded in their results that the MCP-1/CCR2 system plays a role in retinal degradation in rd mouse retinas, and that MCP-1 may not work as a chemotactic cytokine for CCR2 positive monocyte-derived cells in the retina. They also acknowledged previous findings from other publications which reported that "CCL2 or CCR2 deficient mice CCL2 or CCR2-deficient mice develop degenerative retinal changes with age." and a contradictory finding that "CCL2-deficient mice showed no particular retinal change with age." This paper, although performed in mice, shows the most support for the possibility of interaction between Rhodopsin and CCL2 chemokine in the retina. If the mice lacking the chemokine receptor for CCL2(MCP-1) have higher Rhodopsin gene expression, would this not also suggest that the presence of CCL2 may somehow act as a regulator for the Rhodopsin protein?

Publication Source:
https://reader.elsevier.com/reader/sd/pii/S0014483512002710?token=719EC85A116A3A5AAA215C5E68F2CAC95641B08949D06F759AD39BFE7140C69244EF8380F7B43D09F28CFBAA82DE3A3A&originRegion=us-east-1&originCreation=20230517020508

UniProt Information:

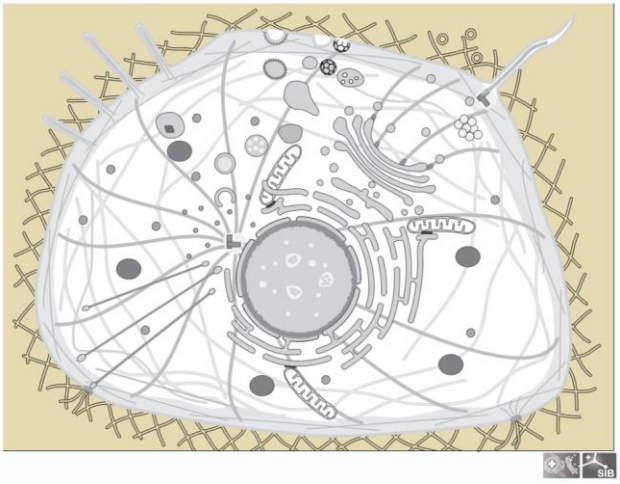
UniProtID: P13500

Molecular Function: cytokine (chemotactic activity for monocytes and basophils)

Biological Processes: Chemotaxis, Inflammatory response

Subcellular Location: Secreted; Extracellular Region; Extracellular Space

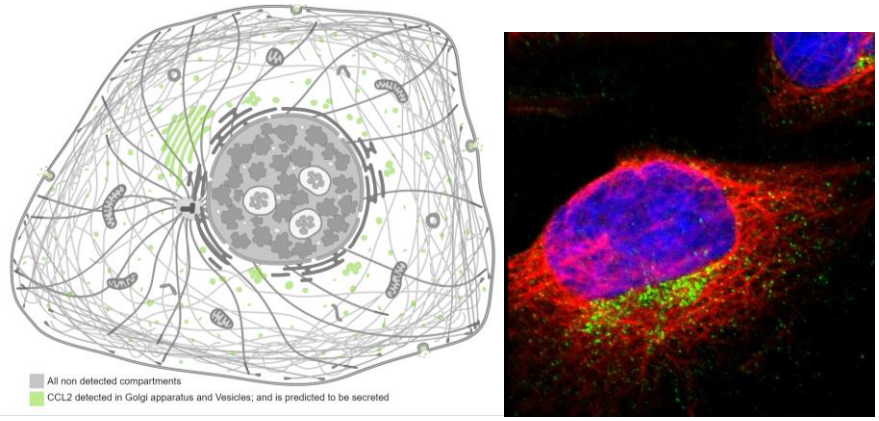
Source: https://www.uniprot.org/uniprotkb/P13500/entry



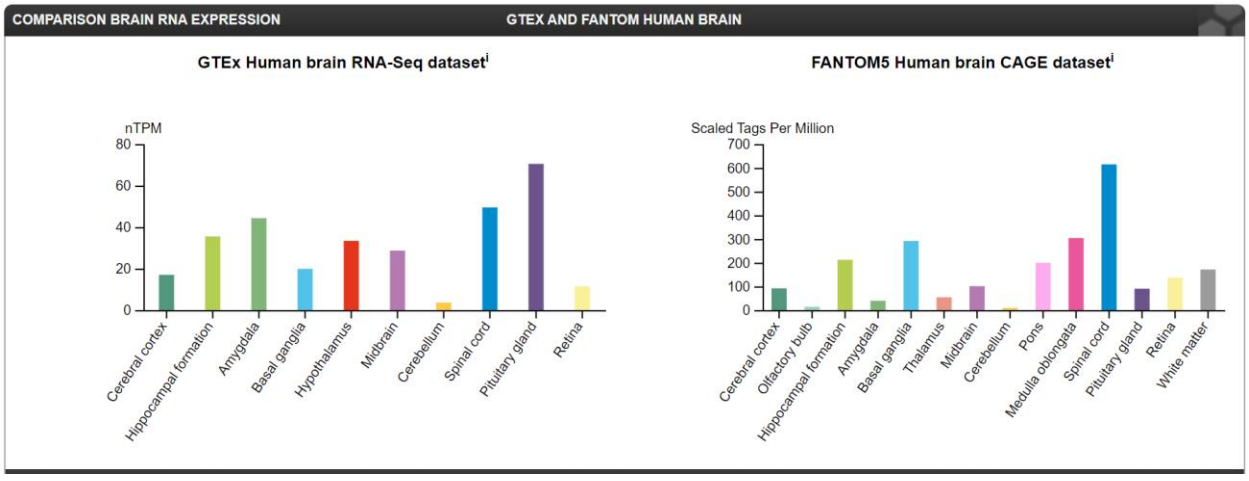
Human Protein Atlas:

Subcellular Location: Golgi, Vesicles, Secreted (predicted)

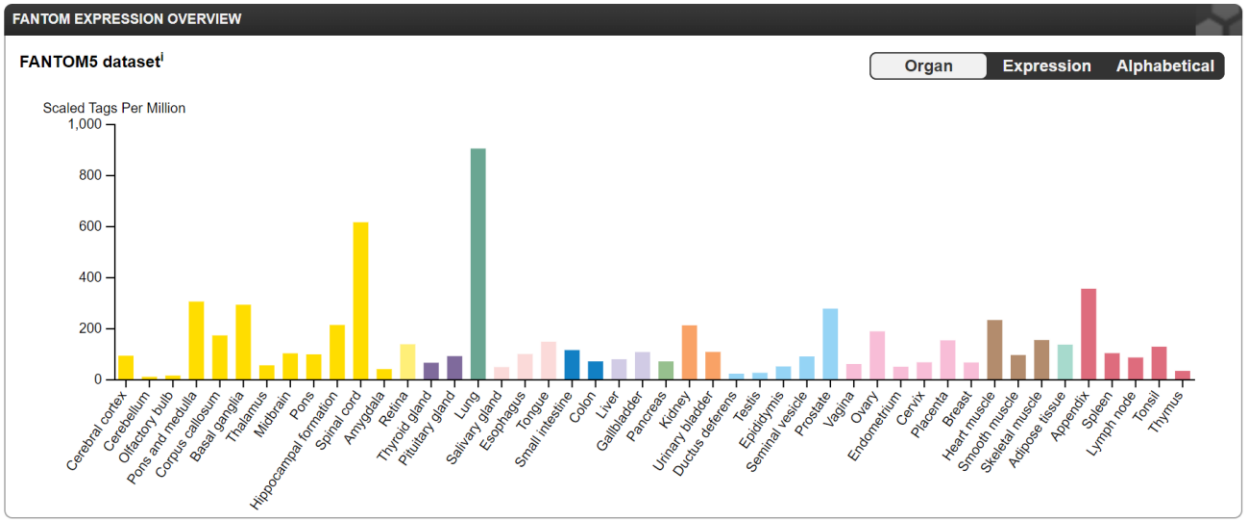
While the subcellular location of this protein was found on the UniProt database to be secreted from the cell, we see similar yet different results from this source. The Human Protein Atlas shows that this protein is found in the Golgi apparatus, vesicles, and predicted to be secreted as seen in the diagram below (left). However, we see an example of expression in U2OS cells (right) labelled as Golgi and vesicle expression with possible secretion. In my professional experience dealing with U2OS cells (Human osteosarcoma) in fluorescence microscopy, Golgi localization of proteins can look very similar to cytoplasmic expression or secretion of the protein, so I am led to believe it could be all of the above. Source: https://www.proteinatlas.org/ENSG00000108691-CCL2/subcellular



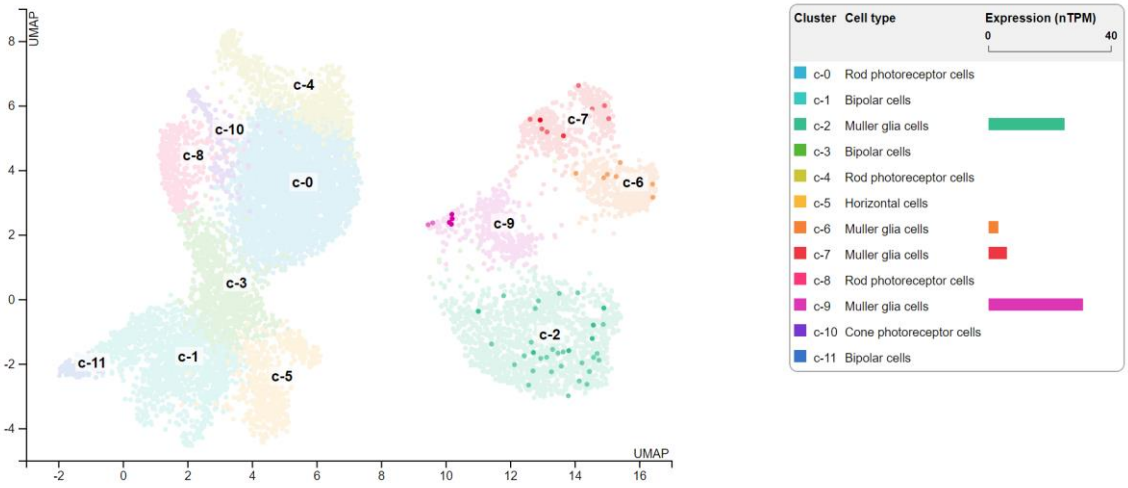
Comparison between Brain RNA-seq dataset (bottom left) and FANTOM5 Human brain CAGE dataset (bottom right) shows expression of CCL2 protein in the retina in both instances. While this expression is relatively low compared to other organs and parts of the brain, it's primary function is immunoregulatory and inflammatory processes. The CC chemokine subfamily is characterized by two adjacent cysteine residues. Acts as a ligand for and binds to CCR2 and CCR4 (chemokine receptors of the same family).



Fantom expression in different tissues in the human body shows that there is expression of CCL2 in the retina. Source: https://www.proteinatlas.org/ENSG00000108691-CCL2/tissue



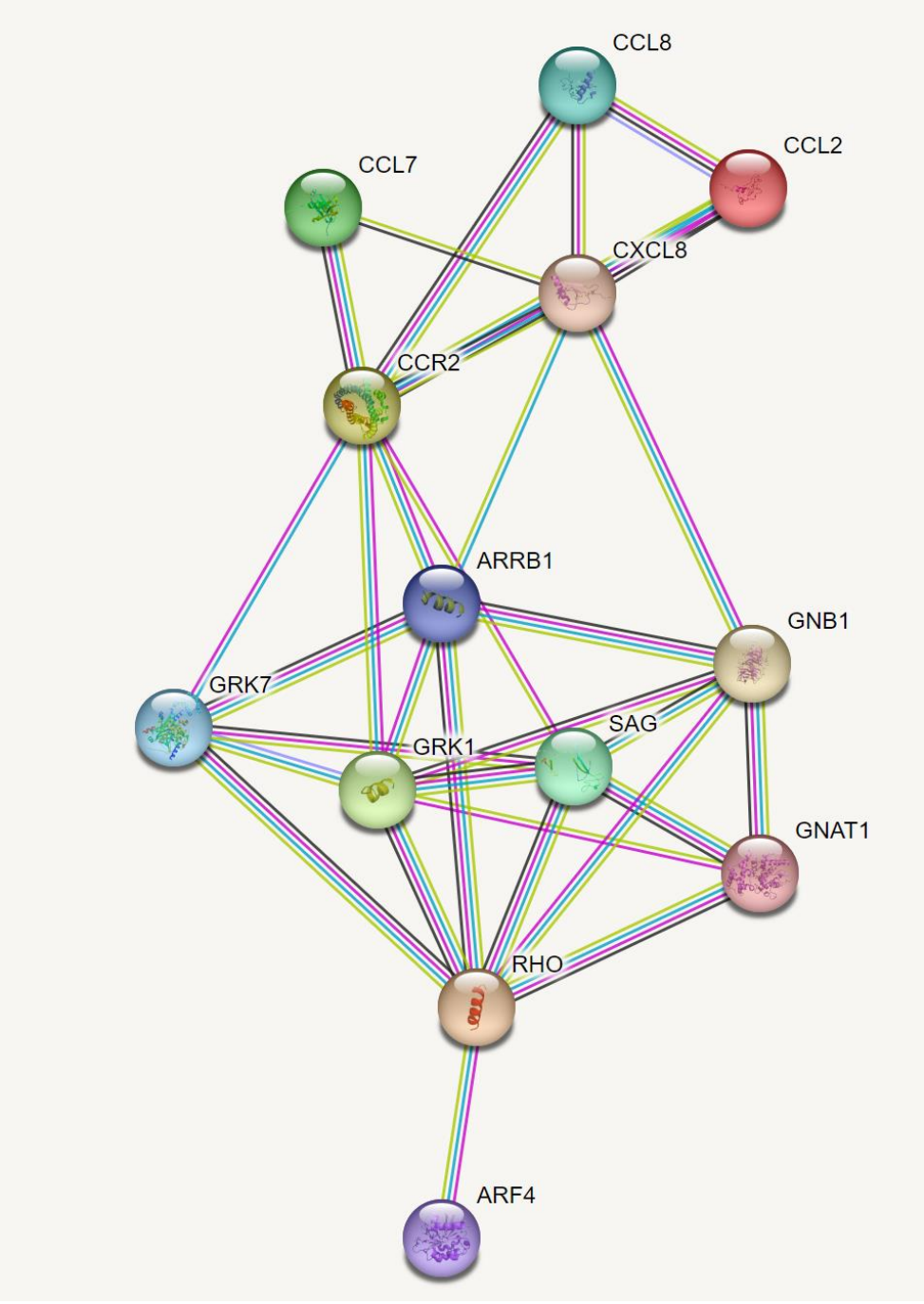
Single Cell Type: If we look at the single cell type under Eye, we can see that the CCL2 expression is predominant in Muller glia cells. Source: https://www.proteinatlas.org/ENSG00000108691-CCL2/single+cell+type/eye



STRING Database:

We can see that CCL2 has no known direct interactions with Rhodopsin. However, we have several known interactions by association. If we trace the blue lines (known interactions) from CCL2 → CCR2 → GRK1(or through ARRB1) → RHO we can see that CCL2 is in the same network as Rhodopsin.

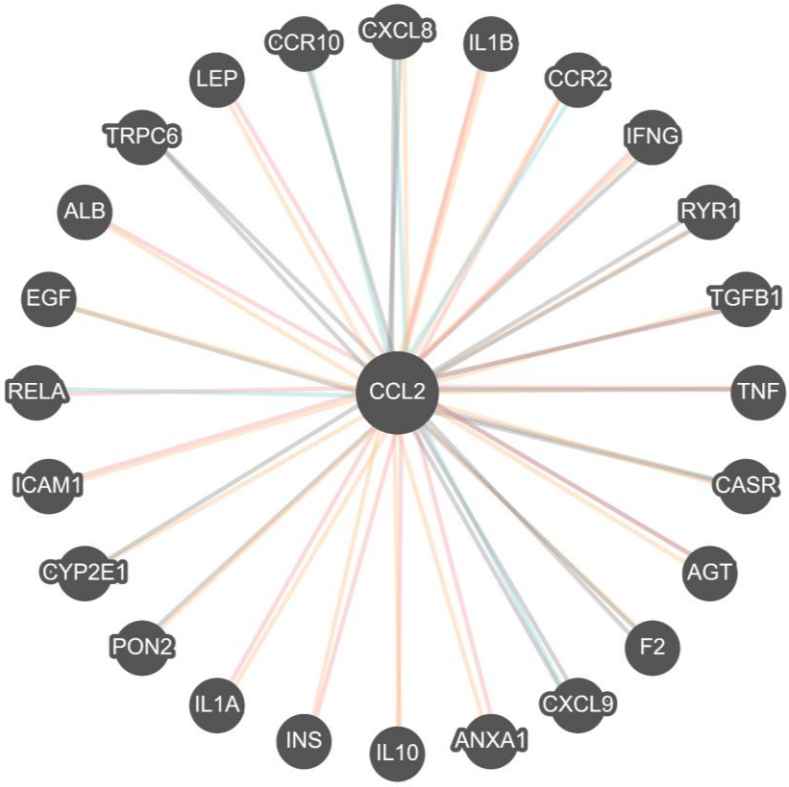
Source: https://string-db.org/cgi/network?taskId=bOoj5YUAWJ3n&sessionId=bf1oTVjMMKvM



Pathway Commons ID: P13500 Source: https://apps.pathwaycommons.org/interactions/?source=CCL2

Accessed through NextProt database link (https://www.nextprot.org/entry/NX_P13500/)

This pathway shows more interactions than the STRING network, based on binding (blue), expression (red), modification (orange), or other (gray). In this network we can see binding, expression, and modification with its known chemokine receptor, CCR2 which also is seen in the STRING network. When compared with the network for Rhodopsin, I did not find any common interactions to CCL2. Although I appreciate the larger amount of interactions listed here, I still prefer STRING network, which is easier to navigate and interact with.



KEGG Database:

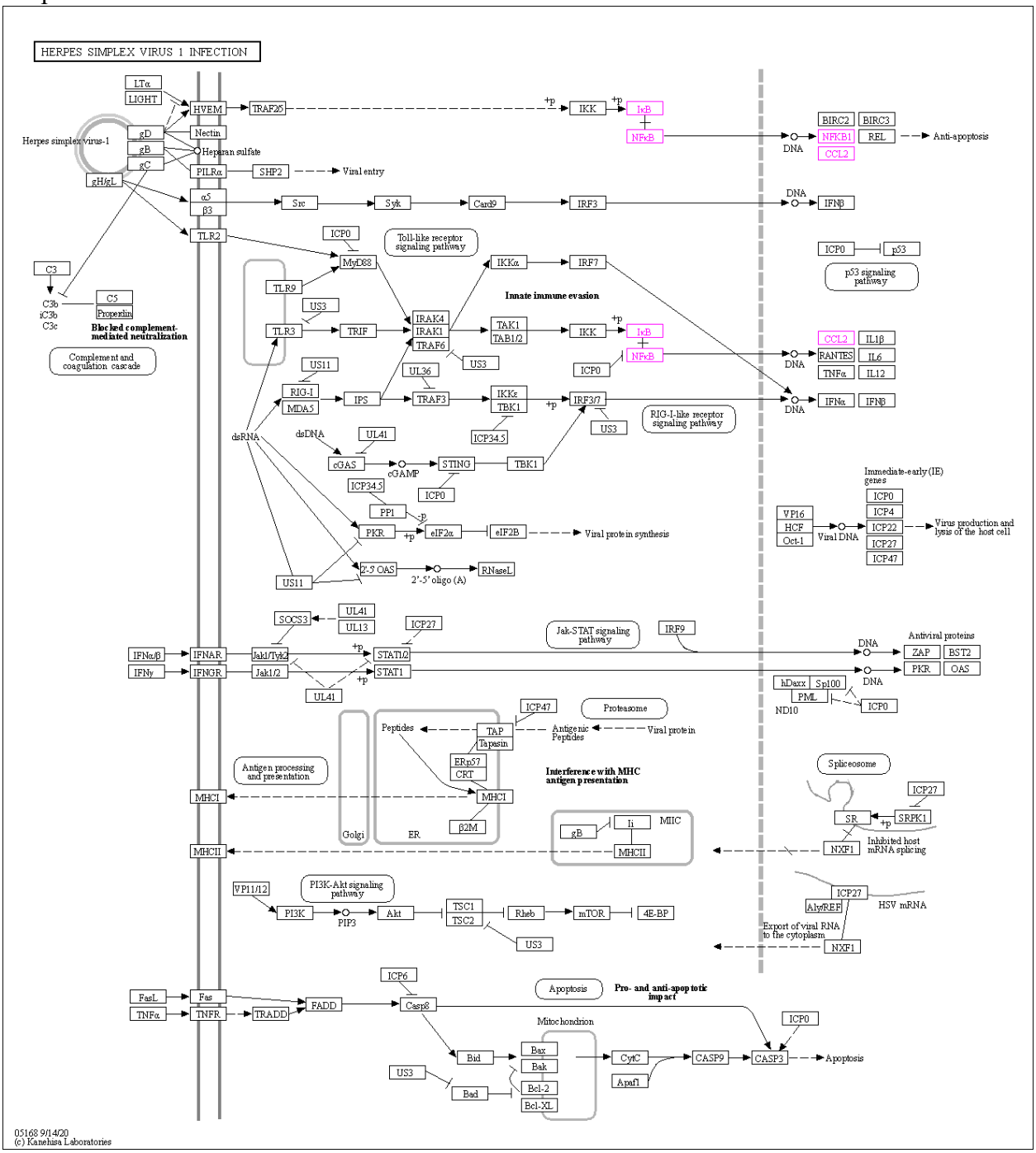
KEGG ID: CCL2 or MCP-1: K14624

KEGG Pathways:

Herpes Simplex Virus 1 Infection (map05168): Source: https://www.kegg.jp/pathway/map05168

I noticed that in this pathway (Below) we have NF-kappaB (a transcription factor that regulates immune responses, inflammation, cytokine production) as well as today's protein of interest, CCL2! Although this pathway is likely not occurring in the retina, it is very interesting to see a protein closely related to rhodopsin near our protein of interest in the same pathway.

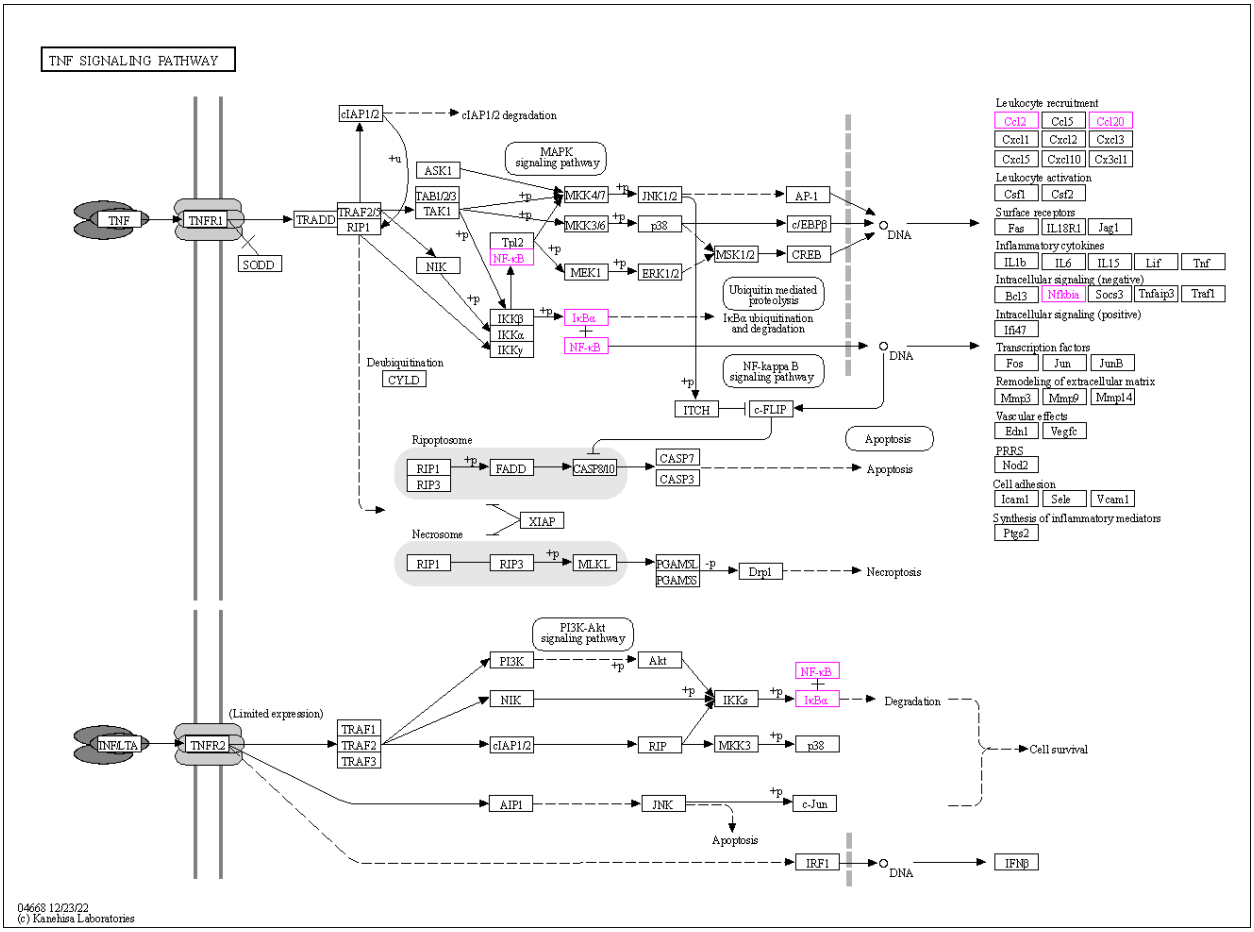
map05168:



TNF Signaling Pathway (map04668): Source: https://www.kegg.jp/pathway/map04668

After seeing that CCL2 was in the same pathway as NFkB, I decided to do a search for both terms and found that they are also both in the TNF Signaling Pathway (Below). It looks like the NF-kappa B signaling pathway is the step between the two proteins.

map04668:



Publications and Other Sources:

Used low-res structures of Rhodopsin as a template for modeling CCR2 (receptor of CCL2).

Publication Source: https://www.jbc.org/action/showPdf?pii=S0021-9258%2819%2962122-8

Discusses CCL2 (MCP-1) relationship to rhodopsin-like GPCR's. CCL2 binding to it's receptor CCR2 has been reported to promote neuroinflammation and maintain Neuropathic Pain.

Publication Source: https://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC8955776&blobtype=pdf

Lists CCL2 PMID's for publications with various GO terms, I was particularly interested in ones with GO term: 0007186 and 0007187 as they relate to G protein-coupled receptor signaling pathways.

Source (QuickGO): https://www.ebi.ac.uk/QuickGO/annotations?geneProductId=P13500