Establishment of a Plasma Cell Culture and Analysis of Expression of Unknown Targets
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Introduction
Plasma cells are a part of the human body’s humoral immune response. During an active humoral immune response, B cells, a subset of white blood cells, differentiate into antibody secreting cells after they have become stimulated by an antigen. In various autoimmune disorders, defective plasma cells secrete autoantibodies which recognize tissues in the body, including the kidneys in lupus and the myelin sheath in multiple sclerosis, as foreign and contributes to the process of damaging those tissues.

During cell differentiation, different proteins are expressed on the cell’s surface which are characteristic of each cell type and stage of differentiation, referred to with the prefix ‘CD’ followed by a number. In humans, two markers used to identify plasma cells are CD27 and CD138. As B cells differentiate into plasma cells they lose CD27 expression and increase CD138 expression; fully differentiated plasma cells lack CD27 and express high levels of CD138. Expression of these markers by the plasma cell lineage is distinct and is used to identify a population of cells as plasma cells.

Materials and Methods
– Cell Culture
  • Myeloma cell lines U266 and H929 were thawed and cultured separately in RPMI medium with 10% FBS in T75 flasks
  • B cells were isolated cells from whole blood from one donor
    – Peripheral blood was diluted 1:1 in RPMI; the mononuclear cell layer was separated out using Ficoll-Hypaque density gradient centrifugation
    – B cells were isolated from mononuclear cells using the EasyStep™ Direct Human B Cell Isolation Kit from StemCell Technologies
    – On Day 0 CD40L and IL-21 were added to culture
    – Cells were cultured in IMDM medium, 10% FBS T75 for 20 days
    – Media was changed on days in which flow cytometry was performed
– Flow Cytometry
  • Cells were stained for 30 minutes with antibody cocktails consisting of:
    – PI/NA4, PE
    – ADRB1, Alexa 647
    – CD39, BV421
    – PRF4, APC
    – CD206, BV421
    – SDC3, APC
    – CD117, BV421
    – PRLR, APC
    – BMPR2, PE
    – CCR2, PE
    – CD27, BV510*FITC
    – CD138, BUV737*
    – CD3, BV711*
    – CD19, Alexa 488*
  • Cells were washed and fixed in 2% paraformaldehyde for 15 minutes in the dark
  • The samples were run in plates on the LSRIII

Table 1. In cell line U266, ADRB1, ENTPD1/CD39, CCR2 and PLXNA4 showed positive expression; in cell line H929, targets ADRB1 and SDC3 showed positive expression.

<table>
<thead>
<tr>
<th>Targets Tested</th>
<th>U266**</th>
<th>H929**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRB1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MRC1/CD206</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ENTPD1/CD39</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCR2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PRF4/CD117</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PRL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SDC3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PLXNA4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BMPR2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*As compared to n-1 isotype control
**As compared to the untreated cells

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Cell Line Analysis - Panel A: Expression of CD138 and CD27
– Cells were labeled with a CD138 PE and CD27 FITC combination stain

Figure 1.
A. U266
B. H929

Figure 2.
A. U266 cell line: Four of the unknown targets, ADRB1, CD39, CCR2 and PLXNA4, showed positive expression in the cell line where the blue curve displays the unstained sample and the red curve displays the stained cells.
B. H929 cell line: Two of the unknown targets, ADRB1 and SDC3 showed positive expression where the blue curve displays the unstained sample and the red curve displays the stained cells.

Figure 3.
A. The expressions of CD138 and CD27 were measured over a 20 Day period in B Cells that had been induced towards differentiating into Plasma cells. B. The expression of the unknown targets were measured in CD19+/CD3- B cells after isolation from a fresh human donor’s peripheral blood sample where the blue curve displays the unstained sample and the red curve displays the stained cells.

Summary
The experiments are being used to drive researchers in the direction of discovering possible antibody targets that are selective to plasma cells. The model of the myeloma cell lines have aided in offering more information about what normal plasma cells are like.

The last phase of these experiments are ongoing with plans to generate fully differentiated long lived plasma cells, as reported in a separate experiment by Cocchi et al. of the University of Leeds (The Journal of Immunology, 2012, vol. 189). Additional cytokines will be included - which have been shown to aid in the differentiation from B cells into plasma cells where a fraction will become long lived. In addition, adherent Mesenchymal Stem cells will be utilized as feeder layer to increase viability of the cells during the long term culture.

Conclusion
The high expression of CD138 and the low expression of CD27 substantiate that the cell lines NCI H929 and U266 are plasma cell lines. Differences in the expression of the unknown targets between the plasma cell lines and the CD19+/CD3- B cell line indicate the existence of these specific proteins on the surface of normal human plasma cells.