IDENTIFICATION OF THE RECEPTOR FOR FGL2 AND IMPLICATIONS FOR SUSCEPTIBILITY TO MOUSE HEPATITIS VIRUS (MHV-3)-INDUCED FULMINANT HEPATITIS

Hao Liu, Li Zhang, Myron Cybulsky, Reg Gorczynski,
Jennifer Crookshank, Justin Manuel, David Grant, and Gary Levy*

1. INTRODUCTION

After MHV-3 infection, susceptible mice develop microvascular disturbances, resulting in intravascular thrombosis and cell necrosis, which correlates with macrophage and endothelial cell production of a unique membrane-associated procoagulant, fibrinogen like protein2 (mFGL2). That FGL2 accounted for pathogenesis was shown by the fact that both neutralizing antibody to FGL2 ameliorated the pathological process and FGL2 knockout mice are resistant to liver disease and have increased survival.[1] First cloned from cytotoxic T lymphocytes, FGL2 was classified as a fibrinogen superfamily member.[2] The procoagulant activity has been localized to the linear N terminal domain of mFGL2. $CD4^+$ $CD25^+$ T cells have recently been shown to secrete a soluble form of FGL2 (sFGL2), which has now been proposed to have immunomodulatory activity.[3] We recently reported that sFGL2 inhibits T-cell proliferation and dendritic cell maturation.[4] That FGL2 exists as both soluble and membrane forms is not unique and has been described for other inflammatory molecules including tissue factor, CD16, and CD38. The purpose of this study is to identify the receptor(s) for sFGL2 and investigate the role of sFGL2 receptor interaction in MHV pathogenesis.

2. MATERIALS AND METHODS

Recombinant protein production: CHO-K1 cells were transfected with a Pires-neo3–Fc-mFGL2 plasmid in which the mouse IgG2a Fc tag was mutated to prevent the binding to FcγR and complement.

* Multi Organ Transplant Program, University Health Network, University of Toronto, Toronto, Canada.

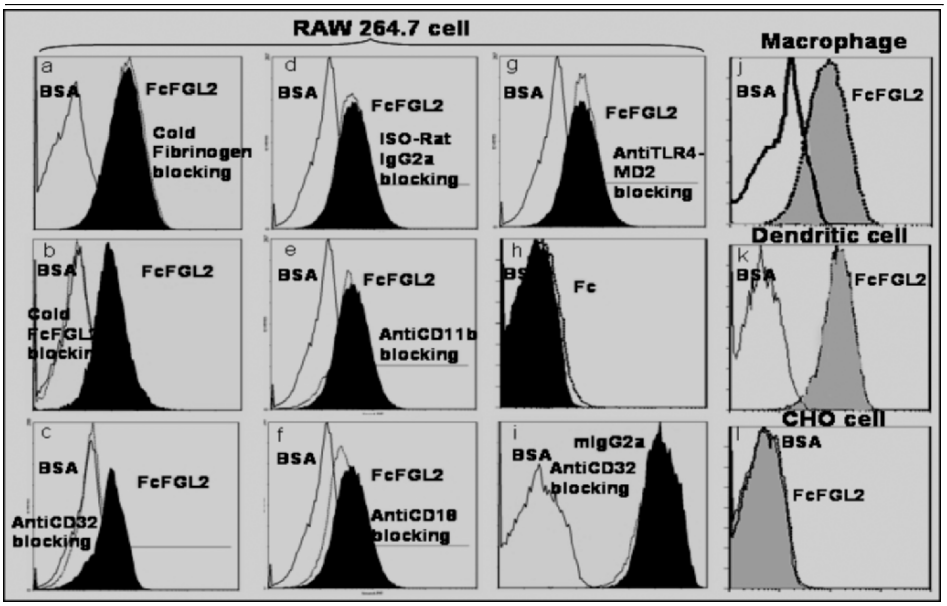

Figure 1. FcFGL2 binding with different cells can be specifically blocked by cold FGL2 and anti-mouse CD16/32.

Receptor positive cells identification: Biotinylated fusion FGL2 was used to study binding to RAW264.7, CHO-K1, peritoneal macrophages, bone marrow derived dendritic cells, and LPS stimulated spleen cells. To test the interaction specificity, 100-fold excess of cold BSA, fibrinogen, and FGL2 were added to compete the FGL2 probe binding.

Strain differences of FcγRIIB allotype: Spleen cells from C57BL6/J and AJ mice were harvested and stimulated with LPS for 48 hours, followed by staining with 2.4G2-FITC, Ly17.1/2-FITC, and anti-CD19-pc5.

3. RESULTS

We first showed that sFGL2 binds primarily to antigen presenting cells including macrophages, dendritic cells and LPS stimulated B cells (Figure 1). Preliminary data indicates that the FGL2 receptor might be one of the low-affinity receptors for immunoglobulin G (FcγR), a receptor family known to link the innate and acquired immunity (Figure 2). Furthermore, sFGL2 bound to B cells from susceptible C57BL/6J mice but not B cells from resistant A/J mice (Figure 3).

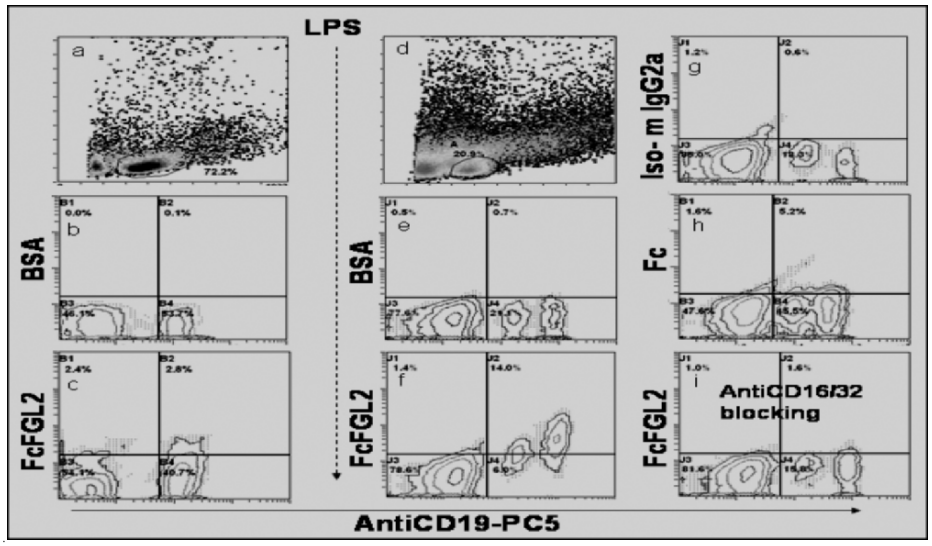

Figure 2. B cell from C57BL/6J binding with FcFGL2 after LPS stimulation can be blocked specifically by anti-CD16/32.

4. DISCUSSION

Here we have identified the inhibitory FcγRIIB as a receptor for sFGL2. As members of the triggering receptors expressed by myeloid cells (TREM), Fcγ receptors consist of both activating (FcγRI/CD64, FcγRIII/CD16 and FcγRIV/CD16-2) and inhibitory (Fcγ-RIIB/CD32) members. There are 2 allotypes of the Fcγ receptors, Ly17.1 and Ly17.2, which are associated with a two amino acid polymorphism in the second extracellular domain.[5] Uniquely, B lymphocytes express only CD32 and NK cells express only CD16 on their surfaces, while most immune cells express all three receptors.[6] The expression level of those receptors can be regulated by Th1/2 cytokines controlling the balance of immune responses.[7]

Viruses have evolved various strategies to counteract host immunity and thus, escape host immune surveillance. For example, coronavirus spike protein (S) displays Fcγ receptor activity. The anti-mouse FcγRII/III monoclonal antibody, 2.4 G2, has been shown to immunoprecipitate S protein of MHV, and by amino acid sequence analysis it was shown that the S protein and the FcγRs share homology.[8] Therefore, it was hypothesized that nonspecific antibody binding with the S protein will prevent antibody-dependent cellular cytotoxicity. This is not unique to MHV, and herpes simplex virus also can induce Fc receptor activity as a means of escaping immune surveillance.[9]

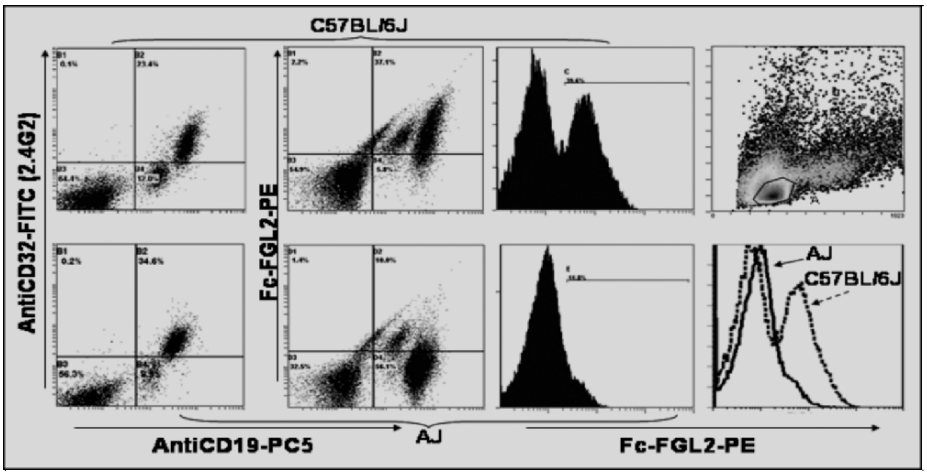

Figure 3. Recombinant Fc-FGL2 bind B cells from B6 but not A/J mice after LPS stimulation.

The potential biological effects of sFGL2 binding to the inhibitory low-affinity FcγRIIB remains unknown at present; however, it may result in inactivation of DC and macrophages and provide yet another mechanism by which MHV escapes immune surveillance. In susceptible mice, the interaction of sFGL2 with the activating CD16 may induce the activation of macrophages, NK cells, neutrophils, and platelets and contribute to disease. In addition, sFGL2 by binding with the S protein, may enhance viral replication. Finally, binding of sFGL2 to B cells may lead to apoptosis resulting in inhibition of neutralizing antibody production. Consistent with this concept sFGL2 does not bind to B cells from A/J mice known to be resistant to MHV, whereas it binds avidly to B cells from susceptible C57BL/6J mice.

5. ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Institute of Health Research (grant no. 37780). Hao Liu is the recipient of a CIHR Regenerative Medicine Fellowship.

6. REFERENCES

1. Marsden, P. A., Ning, Q., Fung, L. S., Luo, X., Chen, Y., Mendicino, M., Ghanekar, A., Scott, J. A., Miller, T., Chan, C. W., Chan, M. W., He, W., Gorczynski, R. M., Grant, D. R., Clark, D. A., Phillips, M. J., Levy, G. A., 2003, The Fgl2/fibroleukin prothrombinase contributes to immunologically mediated thrombosis in experimental and human viral hepatitis, *J. Clin. Invest.* **112**:58-66.
2. Marazzi, S., Blum, S., Hartmann, R., Gundersen, D., Schreyer, M., Argraves, S., von Fliedner, V., Pytela, R., and Ruegg, C., 1998, Characterization of human fibroleukin, a fibrinogen-like protein secreted by T lymphocytes, *J. Immunol.* **161**:138-147.
3. Herman, A. E., Freeman, G. J., Mathis, D., and Benoist, C., 2004, CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion, *J. Exp. Med.* **199**:1479-1489.
4. Chan, C. W., Kay, L. S., Khadaroo, R. G., Chan, M. W., Lakatoo, S., Young, K. J., Zhang, L., Gorczynski, R. M., Cattral, M., Rotstein, O., and Levy, G. A., 2003, Soluble fibrinogen-like protein 2/fibroleukin exhibits immunosuppressive properties: suppressing T cell proliferation and inhibiting maturation of bone marrow-derived dendritic cells, *J. Immunol.* **170**:4036-4044.

5. Schiller, C., Janssen-Graalfs, I., Baumann, U., Schwerter-Strumpf, K., Izui, S., Takai, T., Schmidt, R. E., and Gessner, J. E., 2000, Mouse FcgammaRII is a negative regulator of FcgammaRIII in IgG immune complex-triggered inflammation but not in autoantibody-induced hemolysis, *Eur. J. Immunol.* **30**:481-490.
6. Ravetch, J. V., and Kinet, J. P., 1991, Fc receptors, *Annu. Rev. Immunol.* **9**:457-492.
7. Pricop, L., Redecha, P., Teillaud, J. L., Frey, J., Fridman, W. H., Sautes-Fridman, C., and Salmon, J. E., 2001, Differential modulation of stimulatory and inhibitory Fc gamma receptors on human monocytes by Th1 and Th2 cytokines, *J. Immunol.* **166**:531-537.
8. Oleszak, E. L., Perlman, S., and Leibowitz, J. L., 1992, MHV S peplomer protein expressed by a recombinant vaccinia virus vector exhibits IgG Fc-receptor activity, *Virology* **186**:122-132.
9. Johnson, D. C., Frame, M. C., Ligas, M. W., Cross, A. M., and Stow, N. D., 1988, Herpes simplex virus immunoglobulin G Fc receptor activity depends on a complex of two viral glycoproteins, gE and gI, *J. Virol.* **62**:1347-1354.