Selective Targeting of the FIRE/CIRE Dendritic Cell Surface Receptors Using Hybrid Antibodies:

A New Methodology to Break Tolerance

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Overview of Study

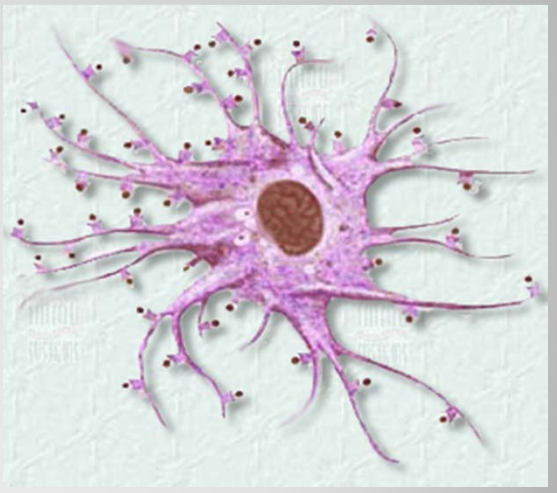
- hybrid mAbs as a vehicle to deliver hAg to DCs is effective at inducing high antibody production
- DC targeting is a new methodology that will ideally be useful in breaking tolerance to proteins that have high homology between mouse and human
- Hybrid antibodies containing the cDNA for FIRE/CIRE DC surface receptors were successfully constructed
- Binding specificity of a-FIRE/CIRE Abs was tested on total lymphocytes and CHO cell lines

The Importance of Dendritic Cells: Background

- Main antigen presenting cells of the immune system

- Responsible for stimulating naive lymphocytes and mount specific immune responses (Steinman et al., 2007)

- DCs are grouped based upon their morphology, localization, surface markers, and functional characteristics (Steinman et al., 2003)



http://www.immusystems.de/assets/images/dendriticcell2.png

Review of Literature

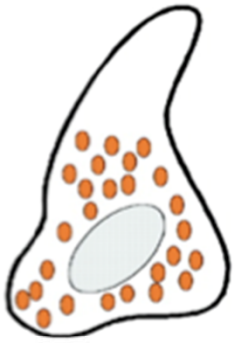
❖Subsets vary in ability to process and present antigens, which is affected by their maturation state (Mellman et al., 2001)

❖Proposed that ability of DCs to elicit both tolerance and immunity is essential in distinction between self and non-self proteins (may be crucial to prevention of auto-immunity) (Boscardin et al., 2006)

❖ TLRs on DC surfaces recognize cognate antigens and stimulate the maturation of cell into cell with prime antigen-presenting capabilities (Banchereau et al., 1998; Janeway et al., 2002)

❖CLR targeting of DCs that mediate efficient uptake of proteins or dying cells can modulate the immune response (Corbett et al., 2005; Hawinger et al., 2001)

Dendritic Cell Maturation



Microbial stimuli

MHC II
lysosomes

Immature DC
Capture of antigens

- absorptive uptake, eg, DEC-205, FcR
- macropinocytosis
- phagocytes: microbes, dying cells

Mature DC
Stimulation of T cell immunity

- CD40, CD86
- CCR7
- IL-12
- High-MHC - peptide

Banchereau and Steinman, Nature 1998, 392:245).

Review of Literature

- by using this antigen targeting method (using hybrid mAbs as delivery vehicle, there is potentially a way to enhance humoral immune response and antibody production (Corbett et al., 2005)

- In this study:
  - "attached" an antigen "X" to the Fc portion of mAbs
  - engineer mAbs to contain a rat F(ab) portion with mIgG2a Fc
  - use hybrid mAbs to target specifically anti-FIRE and anti-CIRE CLRs on total lymphocytes and CHO

Hypotheses

H_1: hybrid mAbs will be successfully designed and produced

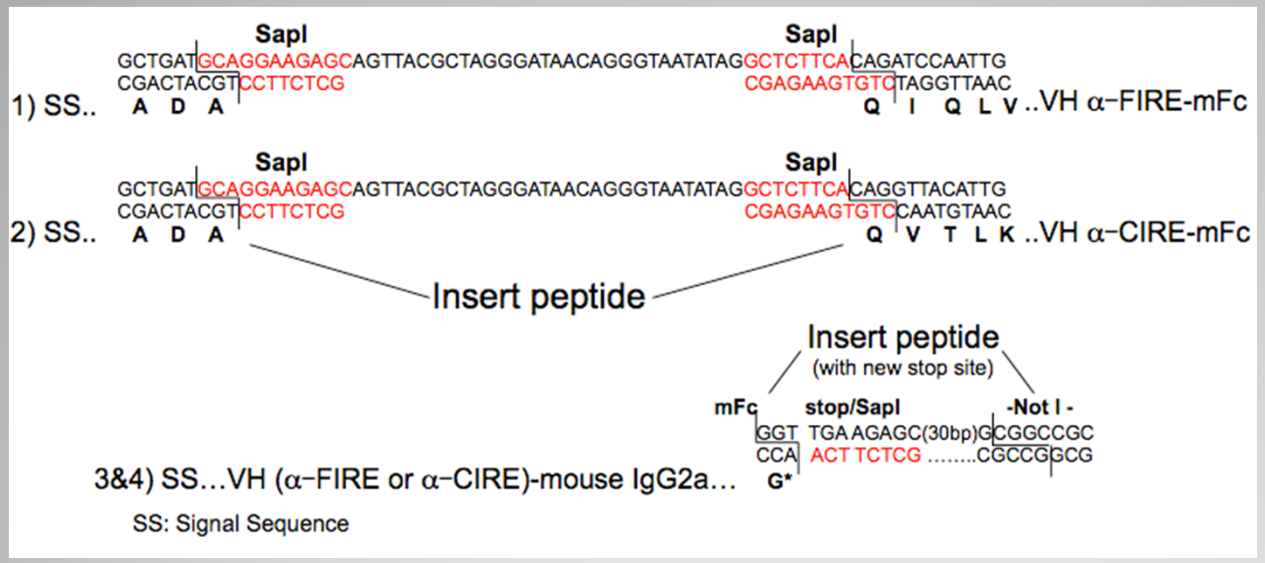
H_2: hybrid mAbs will specifically bind to FIRE/CIRE cell surface receptors when incubated *in vitro* with total lymphocytes purified from C57BL/6 mice spleens and lymph nodes

H_3: hybrid mAbs will demonstrate specific binding to FIRE/CIRE CLRs that are stably expressed on CHO cell lines

H_0: hybrid mAbs will not successfully be produced; however, if they are, they will not successfully bind to FIRE/CIRE cell surface receptors.

Methods

❖ Construct generation:



❖ each variable heavy chain was cloned into REGN's vector on mIgG1a constant region

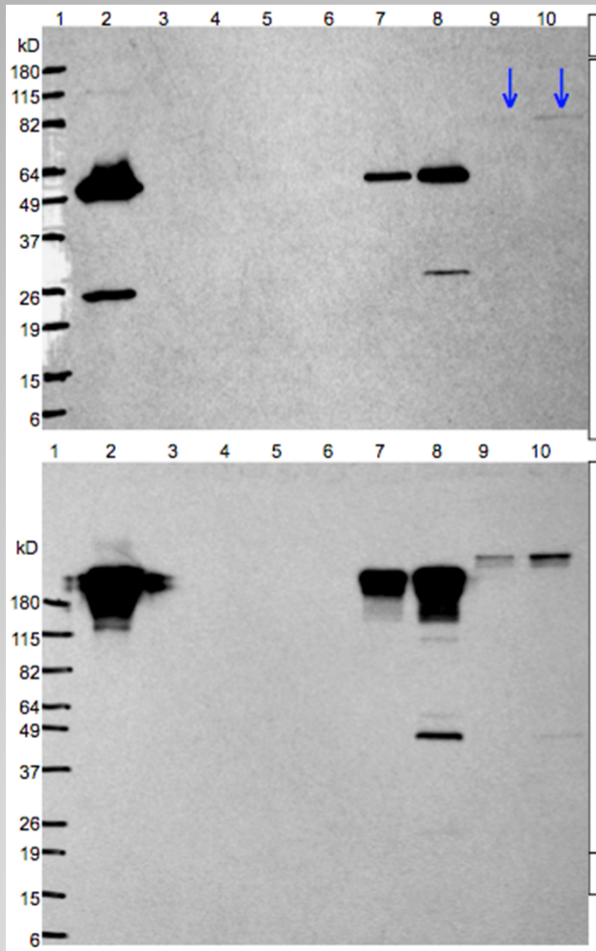
❖ each variable light chain was cloned onto mouse kappa constant regions within vector 3

❖Sub-cloning variable regions onto constant regions resulted in creation of constructs encoding for anti-FIRE and anti-CIRE mAbs

Methods

- hybrid mAb with FIRE/CIRE cDNA were generated by transient transfection of CHO cells using Lipofectamine Plus (Invitrogen) protocol
    - Sample 1: DNA encoding for anti-CIRE mIgG2a
    - Sample 2: DNA encoding for anti-FIRE mIgG2a
    - Sample 3: transfected with 3H7 mock (empty vector control)
- hybrid mAbs submitted for Western Blot Analysis
    - samples separated on SDS-PAGE under reduced and nonreduced conditions
- Binding affinity: purified lymphocytes and labeled with fluorochrome conjugated antibodies→ Checked binding with FACS machine, data analyzed using FloJo software

Data Analysis



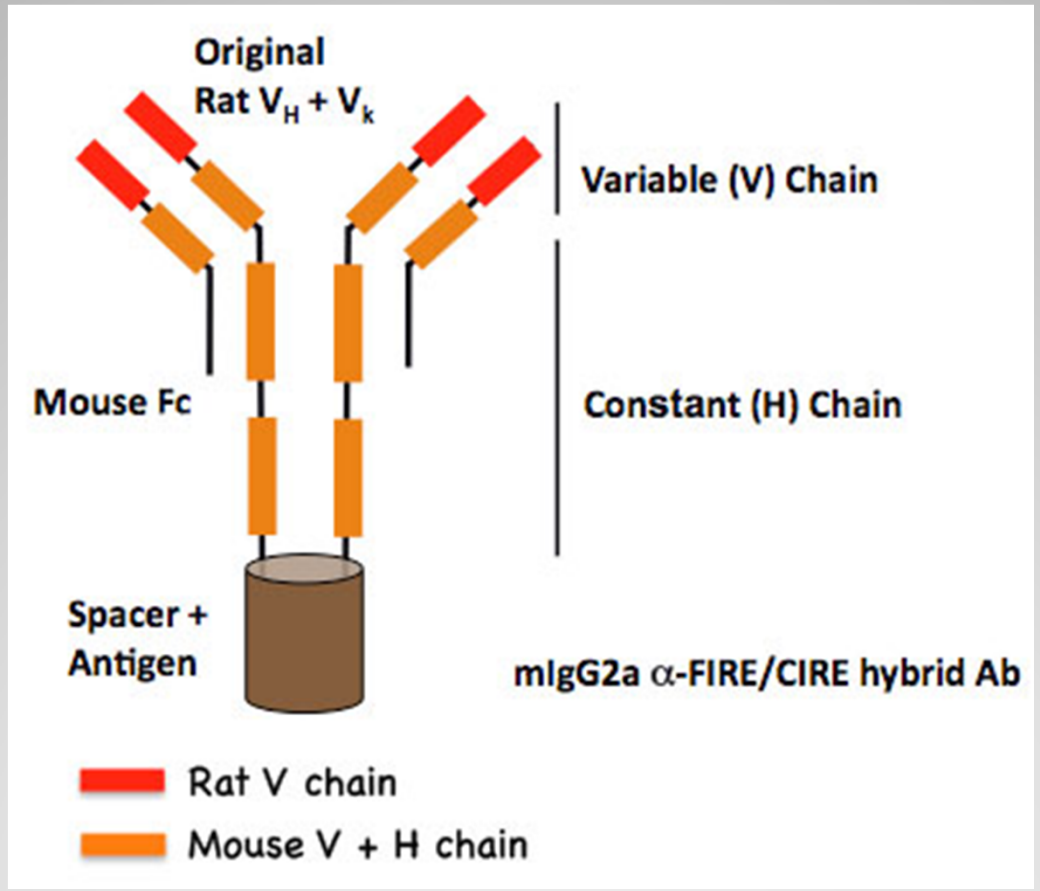
4-20% Novex Tris/Glycine Gel 10 well

Lane	Contents: All Contents are Reduced
1	10µl BenchMark Standard
2	500ng mFc Control Standard
3	32µl MOCK CHO-K1 sup.
4	200ng REGN 910 Anti-hAg mAb
5	200ng REGN 911 Anti-hAng2 mAb
6	200ng hAg from E. Coli
7	32µl Anti-FIRE mAb 6F12 mIgG2a
8	32µl Anti-CIRE mAb 5H10 mIgG2a
9	32µl Anti-FIRE mAb 6F12 w/ Nterm hAg (VH w/ peptide +VK) mIgG2a
10	32µl Anti-CIRE mAb 5H10 w/ Nterm hAg (VH w/ peptide +VK) mIgG2a

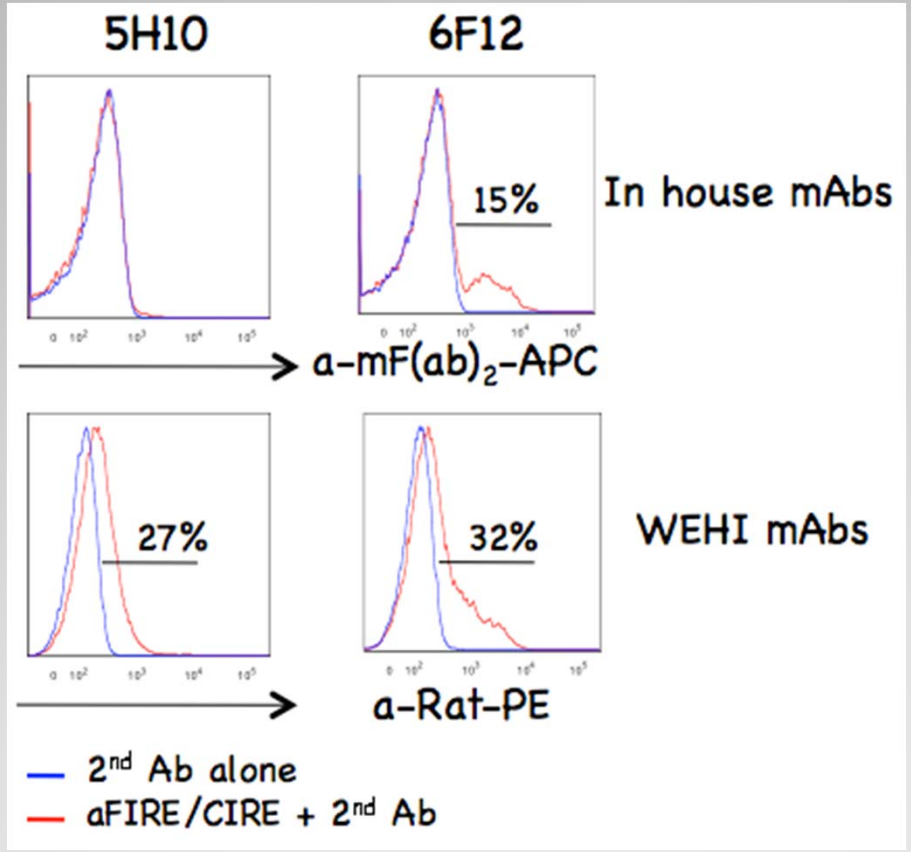
Lane	Contents: All Contents are Non-Reduced
1	10µl BenchMark Standard
2	500ng mFc Control Standard
3	32µl MOCK CHO-K1 sup.
4	200ng REGN 910 Anti-hAg mAb
5	200ng REGN 911 Anti-hAg mAb, VGC 5D10-12
6	200ng hAg from E. Coli
7	32µl Anti-FIRE mAb 6F12 mIgG2a
8	32µl Anti-CIRE mAb 5H10 mIgG2a
9	32µl Anti-FIRE mAb 6F12 w/ Nterm hAg (VH w/ peptide +VK) mIgG2a
10	32µl Anti-CIRE mAb 5H10 w/ Nterm hAg (VH w/ peptide +VK) mIgG2a

- Ab: Pierce anti-mouse IgG-HRP used at 1:15,000 in TBSt
- 30-minute exposure

Results


Original
Rat V_H + V_k
Variable (V) Chain
Mouse Fc
Constant (H) Chain
Spacer +
Antigen
mIgG2a α-FIRE/CIRE hybrid Ab
Rat V chain
Mouse V + H chain

Results of Hypotheses:



Conclusions (Major Findings)

- Binding proved non-specific with splenocytes
- anti-FIRE mAb - 15% binding to CHO cells
- original rat-anti-FIRE (WEHI 6F12) construct - 30% binding
- anti-CIRE mAb - unable to efficiently bind to CHO cell
- Original anti-CIRE (5H10 WEHI) constructs - 27% binding

Discussion

- non-specific binding of mAb with splenocytes
  - could possibly use biotintilated Abs in future to avoid nonspecific binding from species mismatch
- as opposed to 100% rat hybrid mAbs of the past, mAbs used in this study were ¾ mouse IgG2a, and ¼ rat
- Reconstruct anti-CIRE mAbs

Bibliography

• Banchereau, J., & Steinman, R.M., (1998). Dendritic cells and the control of immunity. *Nature Reviews*, 392, 245-252.
• Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M.C., & Steinman, R.M. (2002). Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class i products and peripheral CD8+ t cell tolerance. *The Journal of Experimental Medicine: The Rockefeller University Press*, 196(12), 1627-1638.
• Boscardin, S. B., Hafalla, J.C.R., Masilamani, R.F., Kamphorst, A.O., Zebroski, H.A., Rai, U., *et al*. (2006). Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. *The Journal of Experimental Medicine: The Rockefeller University Press*, 203(3), 599-606.
• Caminschi, I., Lahoud, M.H., & Shortman, K. (2009). Enhancing immune responses by targting antigen to DC. *European Journal of Immunology*, 39, 1-8.
• Corbett, A.J., Caminschi, I., McKenzie, B.S., Brady, J.L., Wright, M.D., Mottram, P.L., Hogarth, P.M., *et al.* (2005). Antigen delivery via two molecules on the CD8- dendritic cell subset induces humoral immunity in the absence of conventional "danger". *European Journal of Immunology*, 35, 2815-2825.
• Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., *et al.* (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *The Journal of Experimental Medicine: The Rockefeller University Press*, 194(6), 769-779.
• Janeway, C.A. Jr., & Medzhitov, R. (2002). Innate immune recognition. *Annual Review of Immunology*, 20, 197-216.
• Mellman, I., & Steinman, R.M. (2001). Dendritic cells: specialized and regulated antigen processing machines. *Cell Minireview*, 106, 255-258.
• Steinman, R.M., & Banchereau, J. (2007). Taking dendritic cells into medicine. *Nature Reviews*, 449, 419-426.
• Steinman, R.M., Banchereau, J., & Nussenzweig, M.C. (2003). Tolerogenic dendritic cells. *Annual Review of Immunology*, 21, 685-711.

Future Research

- ❖ preliminary step in larger study
- ❖ inject constructs into mouse model
- ❖Expectations: very low, if any antibody response to mAb construct itself and high Ab titers against “X”
- ❖Evaluate Ab production: bleed mice and if any anti-”X” Abs are produced, they will be detected by ELISA
- ❖Break tolerance
- ❖Apply to other candidate vaccine proteins that are poorly immunogenic