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Mechanisms Underlying Aggregation Induced Immunogenicity

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Outline

- Aggregate Mediated Immunogenicity Risks
- Platforms/ Tools
- Immune Cell components
- Early vs. late phase immune response
- Size and threshold
- Sequence of the monomer

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Aggregate Mediated Immunogenicity Risks

- Protein based biotherapeutics can particulate/aggregate
- Attributes that can increase immunogenic potential
  - Dose
  - Sequence
  - Immune status and
  - Size and high number of particles
- Clinical Consequences
  - Loss of protein conformation
    - Can induce new epitopes (hypersensitivity)
  - No loss of native conformation
    - Loss of efficacy
    - Cross react with endogenous protein

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Objectives

Evaluate Factors Associated with Aggregate Mediated Risk

- Immune cells and their receptors
- Phase of Immune response
- Sequence of the monomer
- Characteristics (size and threshold)

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Biopharmaceutical Aggregates During All Steps of the Manufacturing Process

Steps During the Manufacturing Process

- Cell culture
- Purification
- Formulation
- Storage
- Shipping
- Administration

Stress Conditions

- Heat
- Freeze-thaw
- Cross-linking
- Protein concentration
- Formulation change – pH, salt
- Addition of extractables/leachables
- Chemical modification
- Mechanical Stress
- Surface effects
- Nano-particles

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Experimental Design

- Challenge mAbs: mAbs1-9
- Generation of Stress Induced Aggregates
  - Stirring
  - Syringe force with silicon oil
  - Heat and pH treatment
- Biophysical characterization
  - Particle counts and size distribution (HIAC)
  - Morphology by Microflow Imaging (MFI)
- *In Vitro* Functional Assays/ *In Vivo* Assays
  - Early Phase (Innate) Assays
    - Whole Blood
    - PBMC
    - Monocyte/DC cell line
  - Late Phase (Adaptive) Assays
    - PBMC derived T cell assays
  - *In vivo*
  - Transgenic mouse models

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BIOPHYSICAL CHARACTERIZATION

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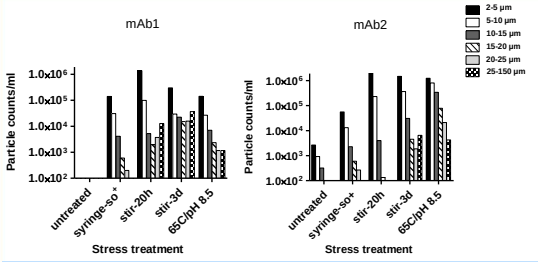
Particle Counts and Size Distribution in mAb Aggregates



IgG$_2$ mAb aggregates have similar aggregate size distribution profiles

*Due to sample dilution and limitations of the instrument, the lower of limit of quantitation was 100 particles/mL.

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Representative Stress Modified Abs

Stress Treatment	Sample Name	Procedure
No treatment	untreated	-
Syringe stress	syringe-so*	Push through a disposable syringe (contains silicon oil) 50x
Stirring for 20 hours	stir-20h	Stir for 20 hours, 2ml in 5cc w/ teflon stirrer bar
Stirring for 3 days	stir-3d	Stir for 3 days, 2ml in 5cc w/ teflon stirrer bar
Heat and pH	65C/pH 8.5	65°C, 10 mM Acetate pH 8.5 for 1hr

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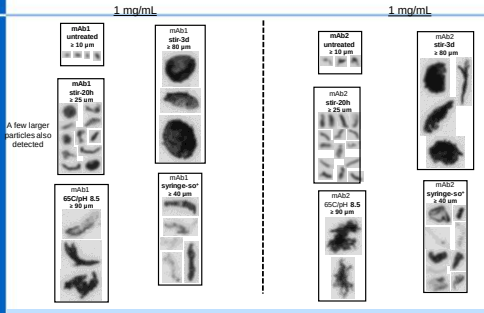
Morphology of mAb Aggregates



mAb aggregates had similar morphologies

Particle images were captured on a liquid-borne particle Micro-Flow Imaging (MFI) System DPA4100.

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IN VITRO FUNCTIONAL *EARLY INNATE PHASE* ASSAYS

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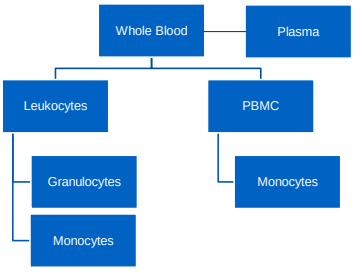
Components of Whole Blood derived Early Phase Innate Response



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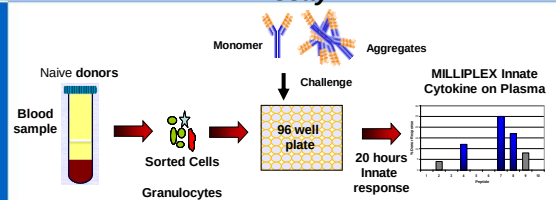
Whole Blood Early Innate Phase Assay



Model for studying cell components of innate phase response

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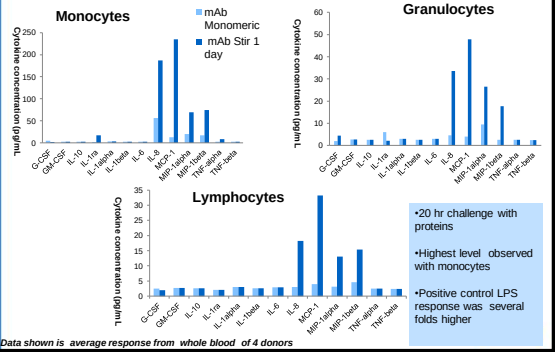
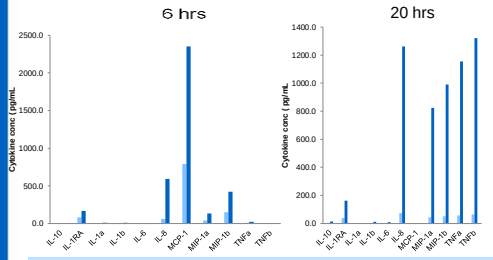
Enhanced Whole Blood derived Cell Response to mAb aggregates



- •20 hr challenge with proteins
- •Highest level observed with monocytes
- •Positive control LPS response was several folds higher

Data shown is average response from whole blood of 4 donors

Whole Blood derived Plasma Cytokine levels following Challenge with mAb Aggregates



Aggregates induced a higher response than monomers
Temporal reduction in the secreted levels
Positive Control LPS response was several folds higher

Data shown is average response from whole blood of 4 donors

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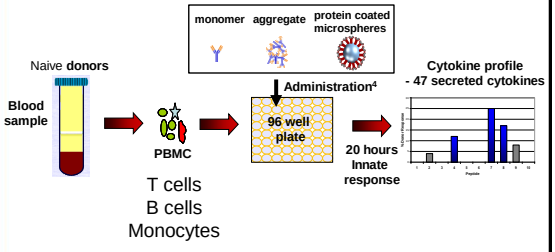
In Vitro PBMC Early Innate Phase Assay



Model for studying monocyte and lymphocyte specific responses

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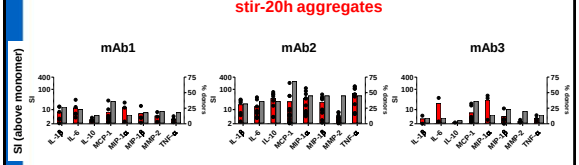
Aggregation of mAbs Enhances the Innate (Early) Innate Response of Human PBMC from Individual Donors (n = 22)



- Results are shown as the average SI of responding donors (above the monomer) and the percentage of donors that responded (n = 22).
- The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

Aggregates made by stirring both IgG_1 and IgG_2 mAbs enhance the response above the response to the monomer.

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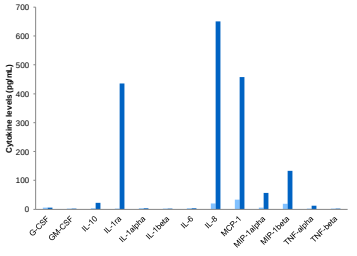
PBMC Response to mAb Aggregates at Early Innate Phase



Monocyte specific cytokine profile associated with aggregate challenged PBMC

The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

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PBMC Response to Aggregates Associated with Distinct Cytokines

Cytokine	*Average SI (above monomer)*	*Average responding donors (%)*	*Conc. range (pg/mL)*	*P-value (*** P < 0.001)*
IL-1β	12 ± 10	23%	1 - 190	***
IL-6	14 ± 14	23%	5 - 12,800	***
IL-10	25 ± 27	18%	1 - 410	***
MCP-1	16 ± 37	47%	360 - 52,700	***
MIP-1α	31 ± 32	24%	110 - 18,600	***
MIP-1β	15 ± 17	29%	320 - 82,000	***
MMP-2	5 ± 2	29%	2,830 - 68,500	***
TNF-α	38 ± 51	26%	4 - 960	***

A distinct monocyte derived cytokine profile is associated with an innate phase response

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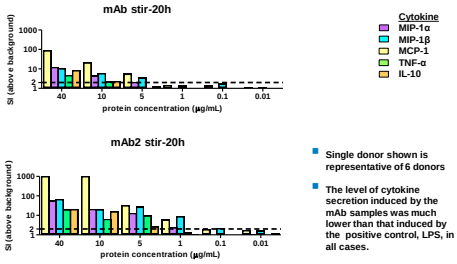
PBMC Release Cytokines in Response to Aggregates in a Dose-dependent Manner



- Single donor shown is representative of 6 donors
- The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

Activation thresholds can be evaluated to a certain extent

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IN VITRO FUNCTIONAL *LATE PHASE ADAPTIVE* ASSAYS

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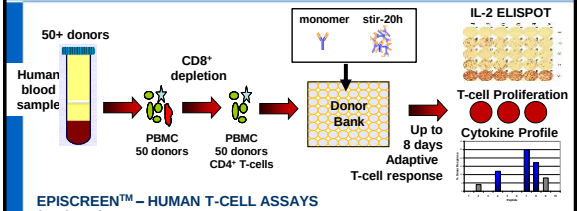
In Vitro PBMC Assay



EPISCREEN™ – HUMAN T-CELL ASSAYS (Antitope)

Biotherapeutic monoclonal antibodies with varying rates of clinical or *in silico* predicted immunogenicity were aggregated, characterized and classified.

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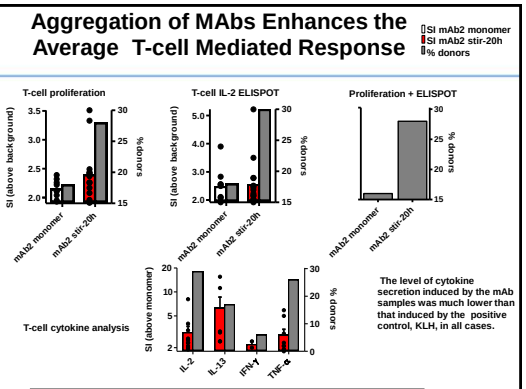
Aggregation of MAbs Enhances the Average T-cell Mediated Response



Aggregates made by stirring induce an immune response that may progress to an adaptive T-cell phase.

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SEQUENCE OF THE MONOMER

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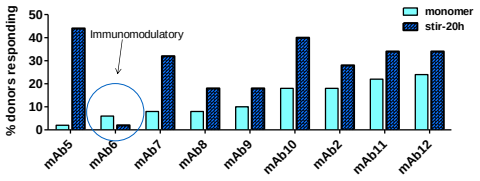
Aggregated mAbs are Associated with Varying Levels of T cell Proliferation



Is sequence of monomer important for aggregate mediated late stage activation?

- Results are shown as the percentage of donors that responded for up to 8 days (n=50).
- Specificity of this response is being evaluated in additional experiments.

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SIZE AND PARTICLE ATTRIBUTES

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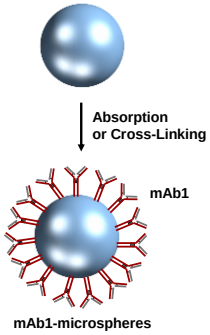
Protein Coated Microspheres were Generated to Control the Size Heterogeneity of Aggregated Samples



Advantages:

- Control the size of the microsphere
- Possibly control orientation of the protein
- Control the number of microspheres injected
- Allows comparison of chemically modified and native protein aggregates

Disadvantages:

- May not be representative of actual protein aggregates
- Structure of protein bound to the microspheres may be altered

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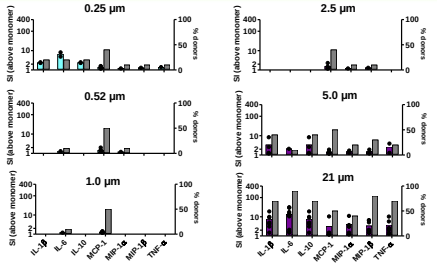
Micron Sized Particles Associated with an Enhanced PBMC Cytokine Secretion



- Results are shown as the average response of responding donors and the percentage of donors (human PBMC) that responded above the monomer (n = 10). SI ≥ 1.2. Samples were tested at an equal concentration of protein.
- The magnitude of the response induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

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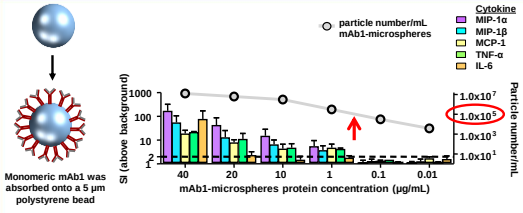
A Dose Response to MAb1-microspheres can be used to Evaluate the Threshold for PBMC Activation



- Results are shown as the average SI (above the background) and standard deviation of a population of human PBMC (n = 2).
- The red arrow indicates the approximate threshold of particle numbers for activation.
- The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

The threshold of activation by mAb1-microspheres in this *in vitro* system is quite high.

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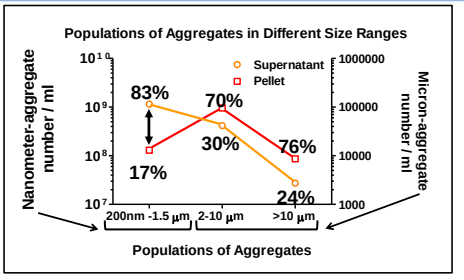
Size-enriched Fractions were Evaluated for Particle Number and Size Distribution



The supernatant fraction is enriched in aggregates in the nanometer range, the pellet fraction is enriched in aggregates in the micron size range.

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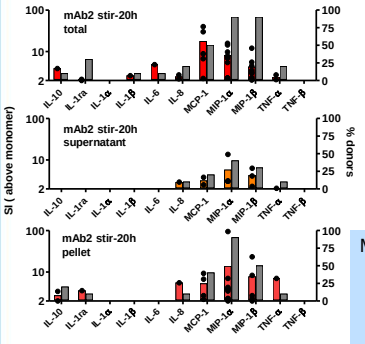
Protein Aggregates in the Pellet Induced a Higher Response in Human PBMC than the Supernatant

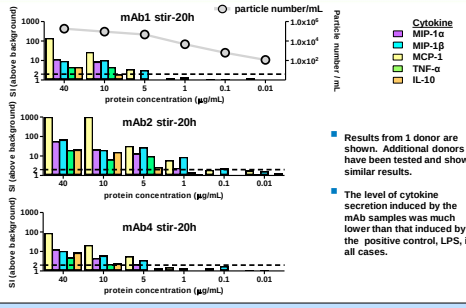


mAb1 was also tested and showed a similar trend.

- Results are shown as the average SI of responding donors (above the monomer) and percentage of donors that responded (n = 10).
- The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.
- PBMC were challenged for 20h

Micron sized particles in the pellet induce a higher activation

PBMC Show a Dose-Dependent Threshold for Activation with Aggregates



- Results from 1 donor are shown. Additional donors have been tested and show similar results.
- The level of cytokine secretion induced by the mAb samples was much lower than that induced by the positive control, LPS, in all cases.

The protein concentration threshold is similar for aggregate preparations and microspheres.

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IN VIVO ASSAYS

Human Antibody Tolerant Mouse

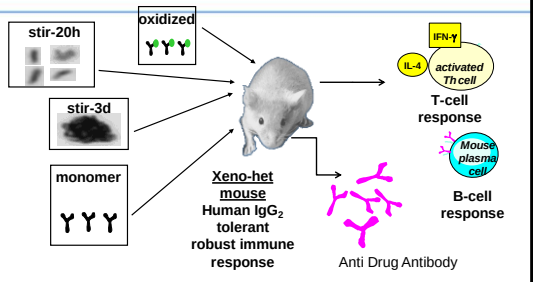
XenoMouse
Human IgG_2 tolerant
weak immune response

X

Wild-type mouse
Human IgG_2 non-tolerant
robust immune response

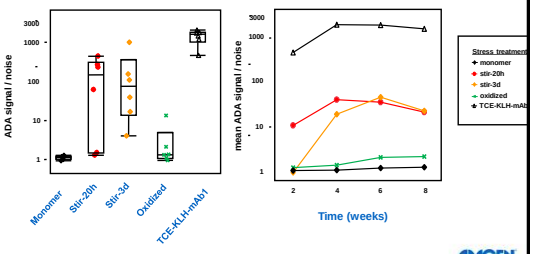
Xeno-het mouse
Human IgG_2 tolerant
robust immune response

Can Aggregates Disrupt Tolerance?



Stress Induced mAb Aggregates Elicited an Enhanced Immune Response in Xeno-het Mice

mAb1 aggregates injected Xeno-het mice at 4-wk (left) and entire study course (right)



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Conclusions

- **Aggregate mediated immunogenicity**
  - Can induce innate and adaptive phase activation
  - Is associated with a certain size and particle number
  - Is associated with a low level ADA response *in vivo*
- **Nature of response is**
  - Weaker than seen with pathogenic stimuli
  - Does not reflect immunogenicity in clinic
- **Immunogenicity risk can be mitigated by**
  - Monitoring of particles in formulations
  - Using in vitro assay systems and in vivo models

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Related publications

- D Wullner, L Zhou, E Bramhall, A Kuck, TJ Goletz, S Swanson, N Chirmule and V Jawa "Considerations for optimization and validation of an in vitro PBMC derived T cell assay for immunogenicity prediction of biotherapeutics" *Clinical Immunology* (2010) 137, 5–14.
- MK Joubert, Q Luo, Y Nashed-Samuel, J Wypych, and LO Narhi "Classification and characterization of therapeutic antibody aggregates" *J Biol Chem.* 2011 286 (28): 25118-33.
- Q Luo, MK Joubert, R Stevenson, RR Ketchem, LO Narhi, and J Wypych "Chemical modifications in therapeutic protein aggregates generated under different stress conditions" *J Biol Chem* 2011 286 (28): 25134-44.
- MK Joubert, M Hokom, C Eakin, L Zhou, M Deshpande, MP Baker, TJ Goletz, BA Kerwin, N Chirmule, LO Narhi and V Jawa "Highly aggregated antibody therapeutics can enhance the *in vitro* innate and late-stage T-cell immune responses" *J Biol Chem.* 2012 287 (30): 25266-79.
- V Bi, V Jawa, MK Joubert, A Kaliyaperumal, C Eakin, K Richmond, O Pan, J Sun, M Hokom, TJ Goletz, J Wypych, L Zhou, BA Kerwin, LO Narhi and T Arora "Development of a human antibody tolerant mouse model to assess the immunogenicity risk due to aggregated biotherapeutics" *Galenics* 2012 in press.

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