

Immunotoxicologic Pathology: clinical biomarkers in non-clinical safety testing

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Society of Toxicologic Pathology-India, October 24 '08

Immunotoxicologic Pathology - inherent in the study of pathology

- And toxicity testing with standard pathology endpoints
- 1970s: recognition of deleterious effects/pathology of pharmacologically potent immunosuppressive agents
- 1979: “*Immunotoxicology*” first used in major scientific publication (*The Immunotoxicology Phenomenon*; Drug Chem. Toxicol.)
 - 1979; Dean, et al.: “*Assessment of immunobiological effects induced by chemicals, drugs or food additives*” (Drug Chem. Toxicol.)
 - Tier testing to screen for immunosuppression (in rodents)
 - **Historical basis for focus on immunosuppression in animal modeling and regulatory guidances**

Immunotoxicologic Pathology - Regulatory Guidelines

- Since 1990s, immunotoxicology (including pathology) assessments have been specifically addressed by all major regulatory agencies

USEPA (OPPTS): *Biochemical test guidelines*, 1996; Health Effects Test Guidelines, 1998

EMA/CHMP(CPMP): *Note for Guidance on Repeated Dose Toxicity Study*, Final July 2000

FDA/CDER: *Immunotoxicology Evaluation of Investigational New Drugs*, Final October 2002

MHLW : *Guidance for Immunotoxicity*, Draft Feb 2003

ICH S8: *Guidance for Immunotoxicity Studies for Human Pharmaceuticals*, Final June 2006

Guidance Comments on Standard Immunotoxicologic Pathology

CPMP Repeat Dose Guidance: *“Information gained in standard toxicity testing can be useful as a primary indicator of immunotoxicity”*

FDA/CDER Immunotoxicology Guidelines: *“Information derived from standard repeat dose toxicity studies can provide early evidence of immunotoxicity”*

ICH S8: Guidance for Immunotoxicity Studies: *“Data from STS should be evaluated for signs of immunotoxic potential.”* ...With emphasis on the following:

*“Hematological changes such as leukocytopenia/leukocytosis, granulocytopenia/granulocytosis, or lymphopenia/lymphocytosis;
Changes in serum globulins that occur without a plausible explanation...”*

Immunotoxicologic Pathology

- the broad definition

Toxicant effects on cells, tissues and/or responses of the immune system detected through pathology endpoints

- *May be morphologic, biochemical and/or functional pathology endpoints*
- *Not necessarily reflective of immunosuppression or immune system enhancement (pro-inflammatory or hypersensitivity response)*
 - **The immune system is complex and redundant -**
Pathology changes are often a mixture of primary, reactive and/or compensatory effects
- *Not necessarily unexpected, unintended, adverse or even undesirable effects*

Immunotoxicologic Pathology - Primary or Secondary?

Differentiating between 1° and 2° effects - key component of interpretation

1° effects

- immunosuppression
- 'immune enhancement'
- immune dysregulation
- immunomodulation

2° effects

- endogenous GCs
- 2° inflammation
- ↓ FC effects
- debilitation

Immunotoxicologic Pathology

Clinical biomarkers in safety testing - Outline

➤ “Tier I” - Routine markers

- *Hematology - Leukogram*
- *Serum Chemistry*

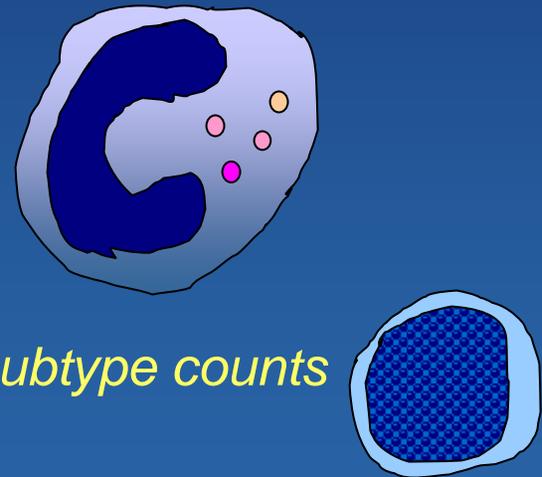
➤ Other Quantitative Chemistry / Immunochemistry

- *Acute phase reactants*
- *Complement, histamine*
- *Immunoglobulin classes*

➤ Phenotypic markers

- *Peripheral blood lymphocyte subtype counts*

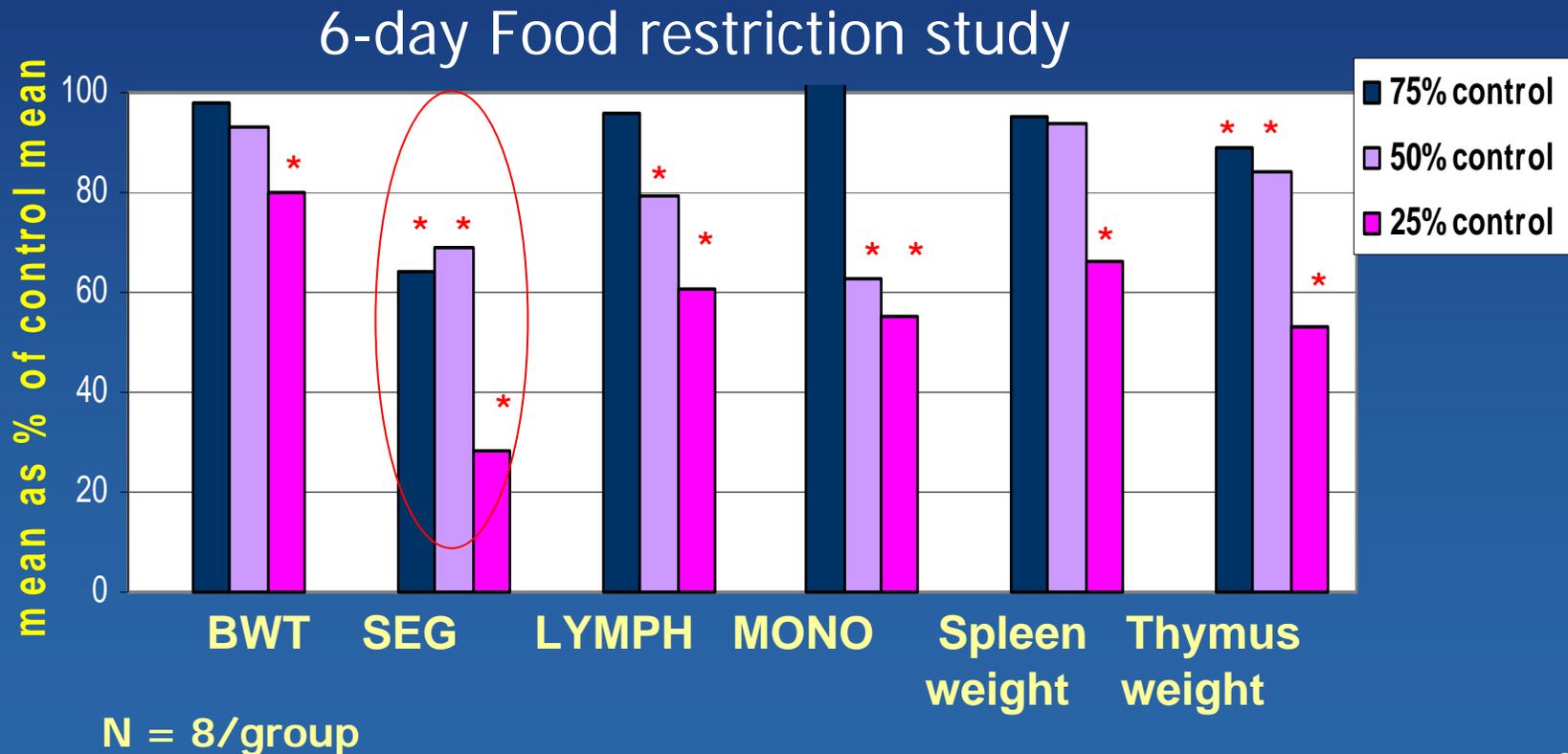
➤ Clinical relevance



Leukogram - peripheral blood leukocyte differential

- **Interpretation - based on absolute counts**
- Species, strain, gender, age differences
- Snapshot of highly dynamic values
 - Plethora of non-toxicologic influences:
 - Handling / acclimation
 - Circadian rhythm
 - Bleeding order
 - Collection procedure, volume(s) and site
 - Fasting/fed & relationship to feeding time
 - Background stress, trauma, infections, anorexia
- **Reference ranges - limited utility**

Food consumption effects on peripheral blood leukocyte counts in male SD Rats



Leukogram - The importance of *patterns*

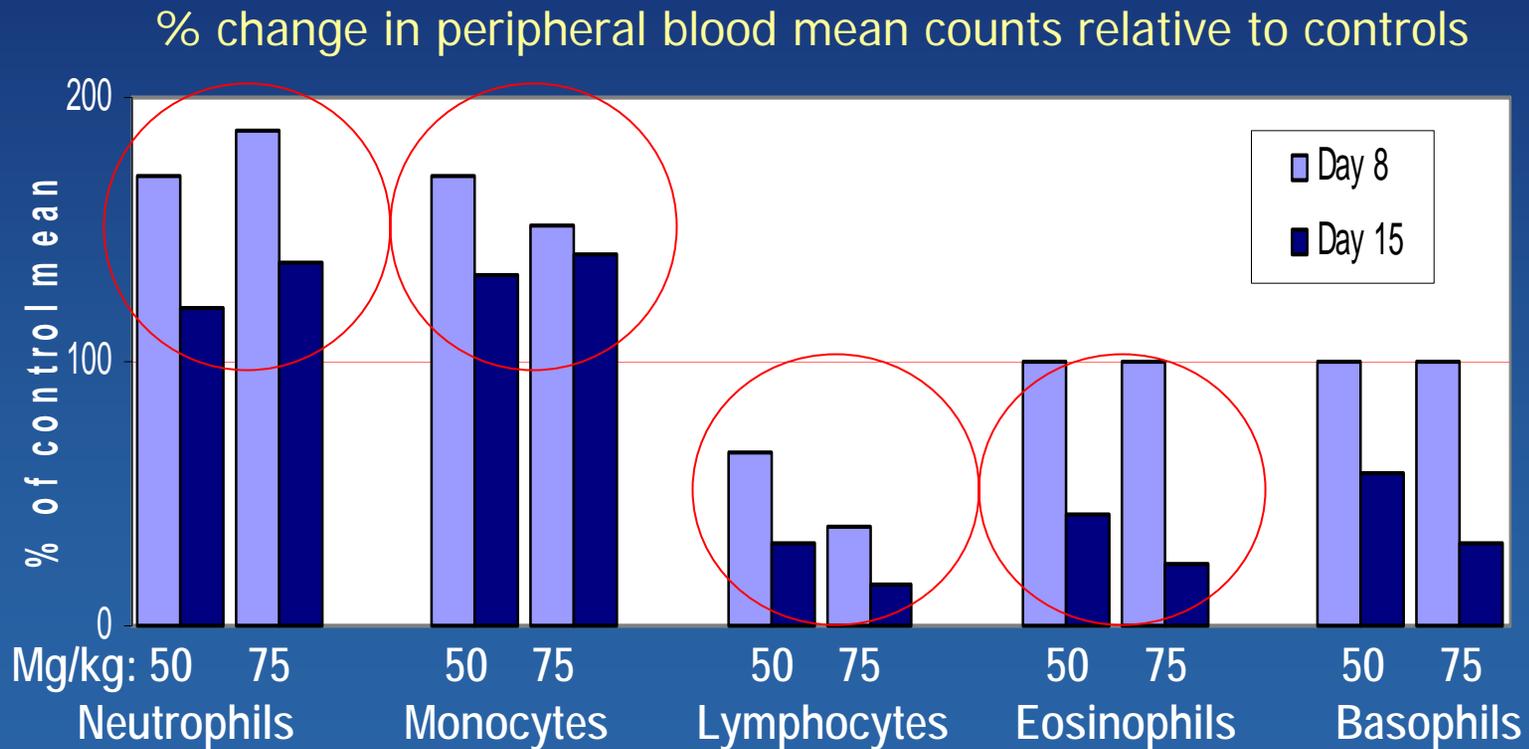
Catecholamine-mediated

- excitement
- vigorous exercise
- CNS / SNS conditions
- hypoglycemia
- acute hypotension
- ↑ Counts of potentially all leukocyte types
- Peracute onset, rapid resolution
- Immature > mature animals
- Monkeys: WBC can readily reach $\geq 20,000$ E3/ μ L

Glucocorticoid-mediated

- ~ “stress” response
- ↓ Counts of lymphocytes, eosinophils (\pm basophils)
- ↑ Counts of neutrophils, monocytes
- Neutrophil & monocyte count changes ameliorate over time
- Histo: \pm adrenal cortical hyperplasia; lymphoid depletion -thymus other

Changes over time in leukocyte counts in rats given a GC receptor agonist



N=15 males/dose group

Leukogram - other common patterns

Inflammation - Acute

- \uparrow - $\uparrow\uparrow$ Neutrophil count, $\pm\uparrow$ monocyte & lymphocyte counts
- \pm Left shift to immature myeloid cell stages; \pm cell 'toxic change'
- Cell counts up to ~20,000 - rats; 15-30,000 E3/ μ L - dog; 20-40,000 E3/ μ L monkey
- $\pm\uparrow$ platelet count - rodents

Inflammation - Persistent

- \uparrow Neutrophil, & often \uparrow monocyte, \pm lymphocyte counts
- Histologic bone marrow, & \pm lymphoid hypercellularity

Others

- Endotoxemia, anaphlactoid/anaphylaxis rxn: \downarrow WBC
- Tissue necrosis, hemolysis: \uparrow *monocyte* & neutrophil count

Leukocyte differential - uncommon patterns

Primary drug-related changes often don't fit these classic patterns

- **Particularly with agents that target specific immune system components, eg:**
 - Adhesion molecule inhibitors
 - Lymphoid cell activation receptor modulators
 - Cytokine and chemokine receptor inhibitors
 - Specific cytokines, growth factors
 - immunoglobulin variants, complement factor inhibitors
 - Acute phase protein synthesis pathway inhibitors

Leukocyte differential - interpretation of uncommon patterns

Consider kinetics of the response (**time of onset, duration to resolution**) to differentiate general mechanism(s): ↓ or ↑

- ← onset
← resolution time
- **Margination** within vessels
 - ~immediately / < 2 hours
 - **Extravasation** from circulation
 - ~hours +
 - **Egress** into circulation through proliferation/
release from origin
 - ~days

Leukocyte differential - interpretation of uncommon patterns

↓ Peripheral blood leukocyte count(s)

← onset
← resolution time

- ↑ **Margination** (eg, $\text{TNF}\alpha$; C5a/C3a generation; liposomal infusions; endothelial cell perturbation; some biomaterials)
- ↑ **Extravasation** (eg, tissue demand > proliferation, IL-1, IL-2)
- ↓ **Egress** from origin (cytostatic, cytotoxic agents, immune-mediated cytopenias, GCs, $\text{IFN-}\gamma$)

Presence or absence of immature cells or morphologic changes can be helpful in interpretation

Uncommon leukograms - consider general kinetics of cell type(s) affected

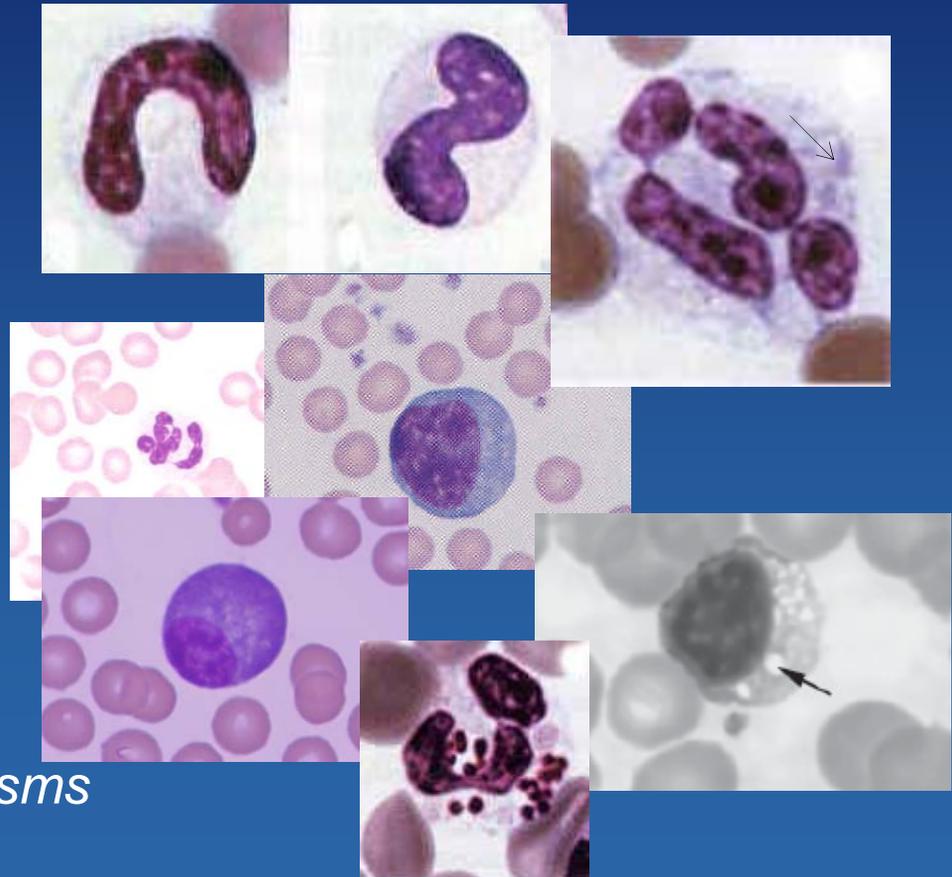
	Primary Site(s) of Origin	Approx. or Avg. Circulation Time	Life Span
Neutrophils	Bone Marrow	6-12 hours	hours → days
Eosinophils	Bone Marrow	3-7 hours ^a	8-12 days ^a
Monocytes	Bone Marrow	1-5 days	days→months
B-Lymphocyte	Lymphoid tissues, bone marrow	days	highly variable
T-Lymphocytes	Lymphoid tissues, thymus	days	variable→years (usually few months)

a: humans; In rodents, circulation time is reportedly up to 24 hours, and peritoneal eosinophils can re-enter circulation

Leukogram -blood leukocyte morphology - as indicator of immune system effects

Most morphologic changes are not specific to the toxicant but may suggest a general mechanism of toxicity

- *Left shift*
- *'Toxic' change (Dohle bodies, basophilia, vacuolation, toxic granulation)*
- *Hypersegmentation suggests prolonged time in circulation; diminished egress*
- *Reactive lymphocytes*
- *Plasma-cell-like morphology*
- *Phospholipidosis*
- *Cytoplasmic inclusions, organisms*



Leukogram - study design, data evaluation - considerations to maximize information

Include 2 or more dosing-phase evaluations

- To assess consistency in the pattern, time-course, correlations with other findings

Evaluate individual values and change over time relative to controls

- **Rodents - compare with age-, gender-, interval- matched controls**
 - Age-related changes complicate inter-interval comparison
 - Consistent method of blood collection
- **Large animals: - compare both magnitude of change and absolute values with that of individual controls**
 - Useful to have ≥ 2 individual pretest values

Questions?



Immunotoxicologic pathology - utility of routine serum chemistry

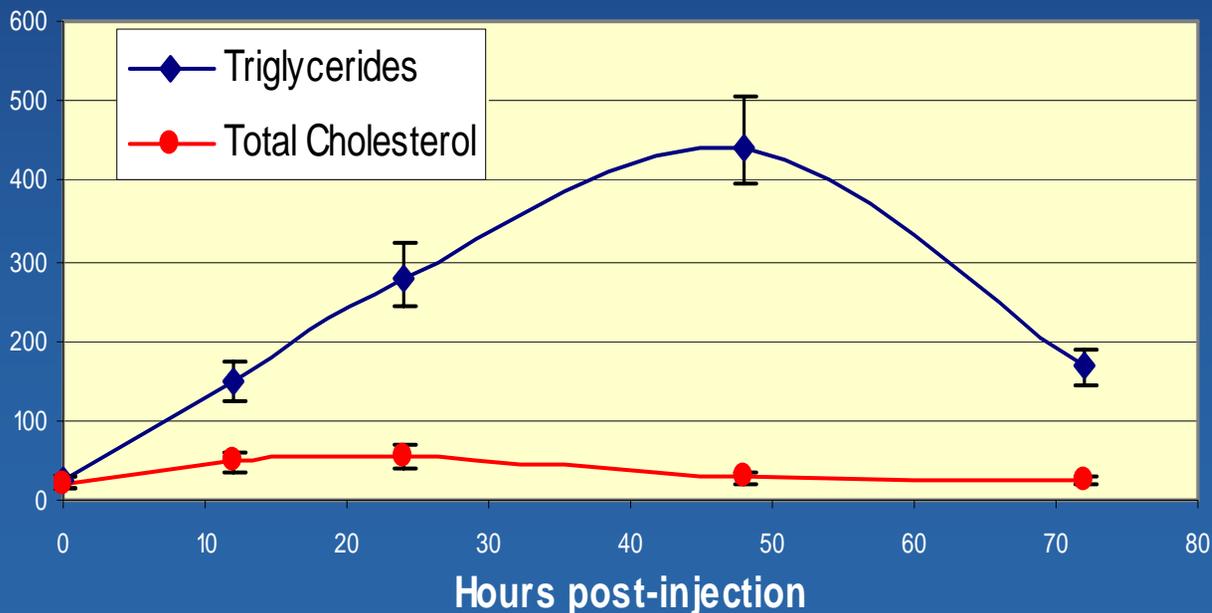
■ Serum Albumin, Total Globulin, Lipids

■ APR of inflammation:

- ↓ albumin
 - ± ↑ total globulin
 - ↑ triglycerides
 - ↑ total cholesterol: rabbit, rat, mouse
 - ± ↓ total cholesterol: humans, NHP
- Onset varies - but all are generally detected in <1 to 3 days of inciting inflammation
 - Some species differences in magnitude and incidence
 - Food consumption changes, other pathology may influence these changes

↑ Triglyceride is most consistent blood lipid change with APR in all species

- 1° Attributed to ↑ VLDLs
- Multitude of cytokines independently induce this change - redundancy in mechanism
- Less profound and consistent total cholesterol change (species-variable, although ↓ HDL cholesterol - all species)



Changes in male rabbits following FCA IM (0.3 mL adjuvant at 3 sites)

N = 6

Immunotoxicologic pathology - utility of routine serum chemistry

Other serum chemistry changes that may represent immune system effects:

- \pm ↓ total globulins with ↓ immunoglobulins (IgG)
 - Serum Ig \approx 40-50% of total globulin in adult animals
- ↓ albumin (and ↑ urine albumin) - with Immune-mediated glomerulonephropathy
 - +/- hypercholesterolemia (nephrotic syndrome)
- Alterations in lipids, glucose, electrolytes with some immune-mediated endocrinopathies
 - Findings vary with gland(s) affected, extent of effect on hormone production/secretion, and species

Consider the combination of physiologic processes that influence routinely-measured serum proteins and lipids in interpretation



Serum Acute Phase Reactants (APRs) as markers of inflammation

APRs are produced by hepatocytes - some also by other cells (eg, macrophages, megakaryocytes) in response to proinflammatory cytokines

- More **specific** to systemic inflammation than hematology or routine serum chemistry parameters
- Some are also more **sensitive** to cytokine-triggered inflammation to allow early detection of onset and resolution
- Reliable assays with acceptable precision and robustness; some can be automated; ~**stable** in serum/ plasma samples
- Utility as bridging biomarkers - comparable application and analyses in clinical testing

Serum APRs as markers of inflammation

Induced or down-regulated by major cytokines:

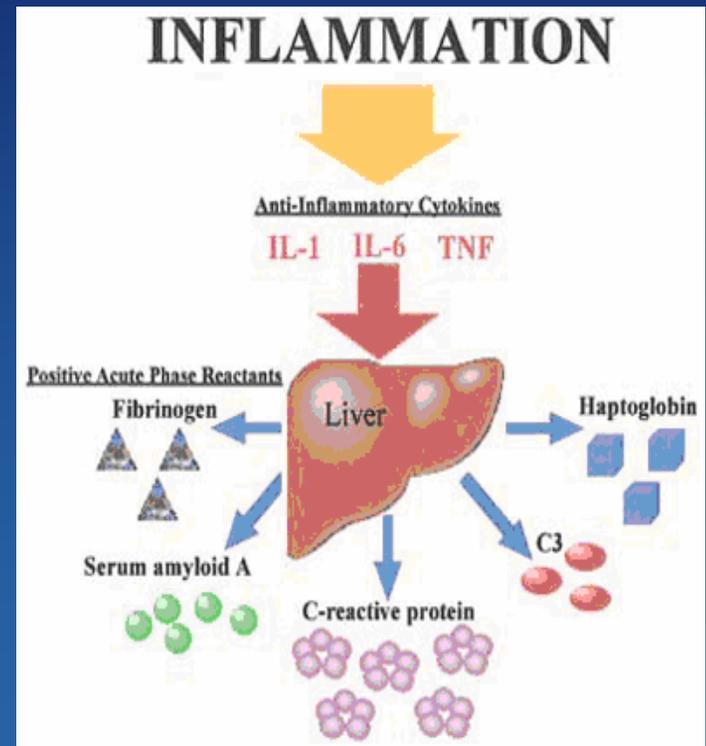
Induced especially by: IL-1_{α/β}, IL-6, TNF_{α/β}

Major modulators: TGF-β, glucocorticoids, insulin

Cytokine signaling → altered nuclear receptor expression (eg, PPAR, LXR, RXR, CAR, PXR)

APRs have wide variety of functions in association within inflammation, eg:

- **Tissue repair and remodeling role (fibrinogen, SAA)**
- **Leukocyte activators (CRP, C3a)**
- **Transport proteins (haptoglobin)**



Serum APRs - Selection for testing on study

Specificity of some APRs to certain *clinical* disease states

- Major patterns:

Type 1: IL-1 and IL-6 for maximum synthesis

- eg, of CRP, SAA, α 1acid GP

Type 2: IL-6 only for maximal induction

- eg, of fibrinogen, haptoglobin, and α 2MG

Additional differential, additive, synergistic, and antagonistic APR induction occurs between mediator substances

Influences of certain other pathologic conditions: hemolysis and haptoglobin; lipid metabolism and SAA; coagulation and fibrinogen - on circulating concentrations

- **Selection of specific APRs in non-clinical testing -1° based on those appropriate for the species**

Species differences in utility of classic APRs as circulating markers:

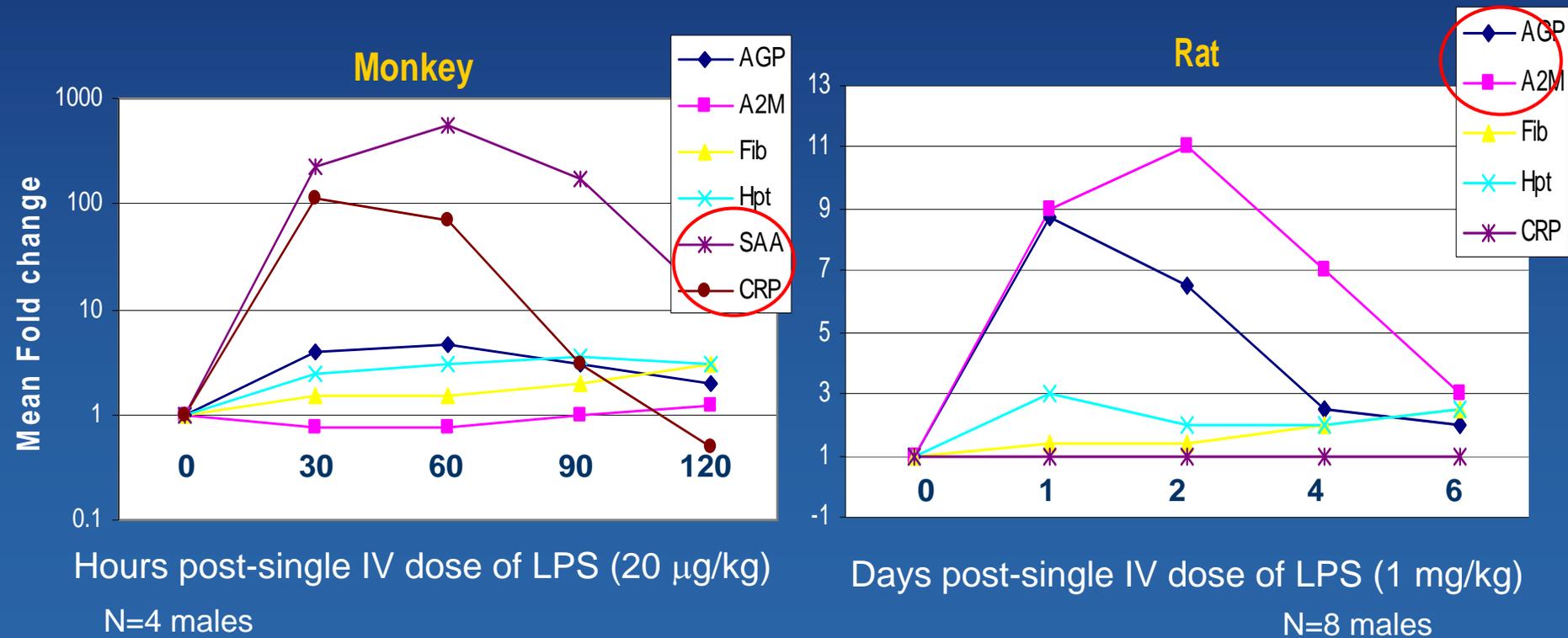
Species differences in APRs detectable in blood, and their responses

APR:	human	dog	rat	mouse	Rabbit	Monkey
CRP	++++	++++	0	+	++++	++++
SAA	++++	++	0	++++*	++++	++++
SAP	0	0	0	+++	?	0
α 2MG	0	+/-	++++	+/-	++	0
α 1acid GP	++	++	+++	0	?	++
ceruloplasmin	+	+	+	?	++	+
haptoglobin	+	++	+++	++	+++	+
fibrinogen	++	++	++	++	+++	++
Transferrin	↓	±↓	±↓	↓	↑	↓
↓alb	√	√	√	√	√	√

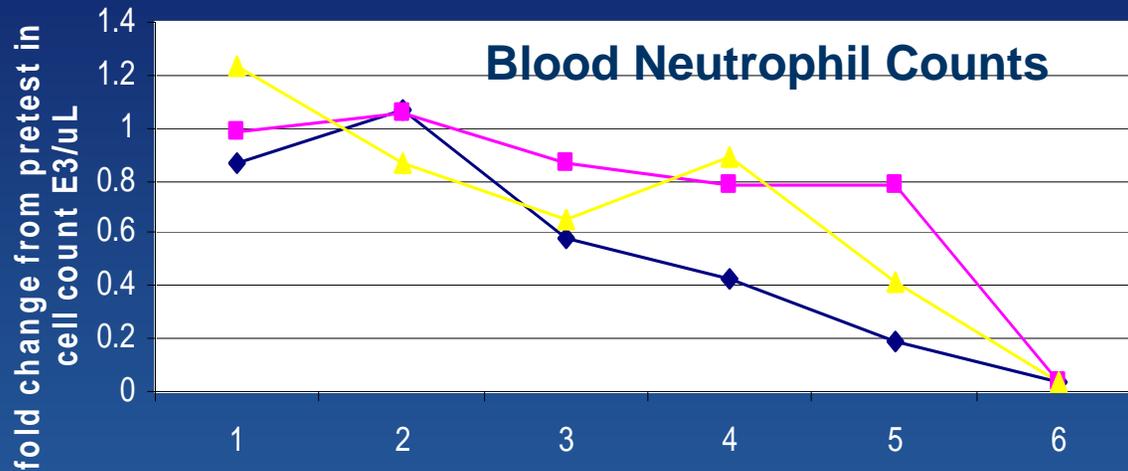
+/- variable results; * strain-dependent + <1x, ++ 1-4x, +++ 5-10x, +++ >10x

Kinetics of APRs with inflammation

Fold change in serum APRs to LPS - monkey and rat

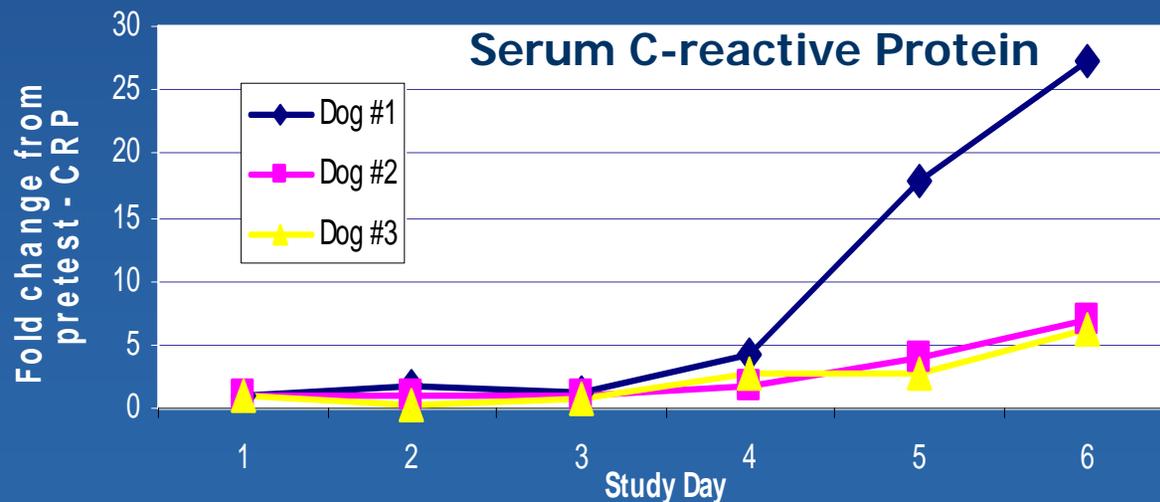


Use of APR's to identify inflammation in dogs given cytotoxic/cytostatic drug



3 male dogs administered high dose of test agent daily.

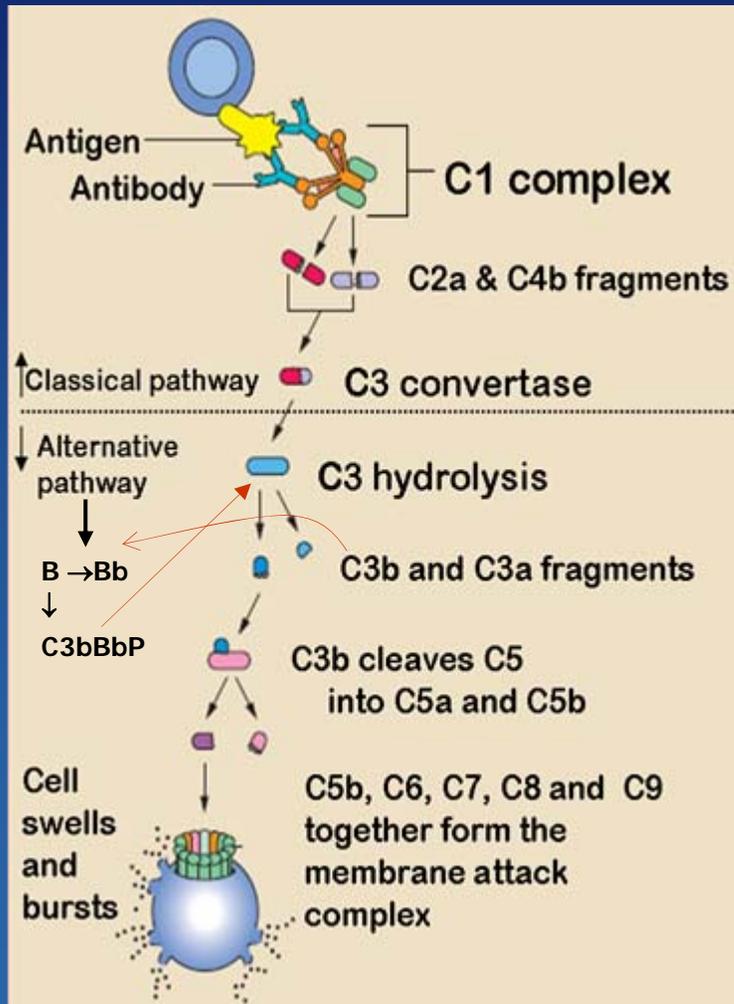
Marrow hypocellularity and hepatic sinusoidal inflammation and were observed, histologically (Day 6).



APRs as markers of inflammation study design/ data interpretation

- Sensitive to small changes in AP state
 - Preferable to evaluate at multiple intervals
 - Utility in determining kinetics of inflammatory process
 - eg, evaluation of vaccines/ adjuvants, anti-inflammatory efficacy, toxicity onset/ resolution
- Most assays are species-specific
 - Exceptions to this are generally less sensitive and specific APRs to inflammation (ie, fibrinogen, haptoglobin)
- Stable - even with prolonged freezer storage
- Specific APRs may also be useful to evaluate other pathology - coagulation (fibrinogen), hemolytic conditions (haptoglobin), hypersensitivity (complement components -C3, C4)

Complement (C') Components



Some components (C3 & C4) are APRs

More commonly assessed in non-clinical studies to determine:

- Involvement in hypersensitivity or immune-complex disease
- To screen for activation
 - Classical pathway
 - Ig-inducing Abs, vaccines or cytokines
 - Alternative pathway
 - Biomaterials, oligonucleotides, vehicles, etc. (alternate pathway)

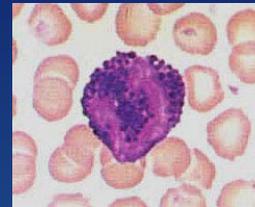
Both pathways can lead to →

- microbe opsonization; cell membrane attack complex, pro-inflammatory effects

Complement-mediated hypersensitivity



C5a, C3a



Direct & indirect vasoactive effects

Basophils, mast cells, eosinophils, neutrophils, macrophages, endothelial cells and small subset of T cells have receptors for C5a, C3a

May see: peripheral edema (eg, facial / periorbital), CNS suppression

Central hypotension, bradycardia or tachycardia, dyspnea

↑C5a and/or C3a, ↓C3, ±↑ plasma histamine

Peracute ↓ neutrophil count followed by ↑ (± left shift); ↓ platelet count; ±↑Hct (hemoconcentration)

Measuring C' components may further distinguish at which dose(s) activation occurs, and whether alternative or classical pathway is predominantly involved

Complement testing - assay methods

Functional bioassay for total serum activity : CH50

- Sample dilution at which 50% lysis of antibody-sensitized RBC (or liposomes) in solution is observed
- For all species - though some species variability in results, assay requirements
- Results highly dependent on assay conditions and procedural consistency

Commercial immunoassays:

- Species-specific assays for C3: mouse, rat, GP, dog, monkey
- Species-specific assays for C3a (adesArgs): dog, monkey
- Some human immunoassays with NHP: C3a, C4a, C5a, Bb
- Novel flow cytometry assays: membrane-associated C' products

Sample handling to avoid in vitro activation; avoid freeze/thaw; consider plasma for analysis of split products (C5a, C3a, C4a, Bb, etc)

Complement testing - assay results

Sample collection intervals: 1) bracket peak effect, PK exposure or Ig response, if possible

↓ **CH50, ↑C3a, C5a, C4a and/or Bb suggests C' activation**

- ↓ **CH50 supports C' component consumption**
- ↑**C3a, C5a verifies ↑split product generation (in vivo/ in vitro)**
- **Alternate pathway activation (↑Bb)**
- **Classical pathway activation (↑C4a)**

↓ **CH50, C3 & C4 may also occur with**

- Malnutrition
- Septicemia
- Recurrent microbial infections
- Autoimmune, and immune complex diseases
- Glomerulonephritis, membranous nephritis

↑ **C3, C4:** Acute phase response



Histamine - evaluation for role in hypersensitivity reactions

1° Mast cell/ basophil/ eosinophil activation via:

- **Anaphylactoid:** membrane “destabilization” or secretagogues -
1) osmolar or ionic effects on cell membrane; 2) receptor-independent activation of G proteins; 3) receptor-mediated G-protein activation
 - *Examples: Compound 48/80, Polymyxin B, dextran (rats), and (dogs) Polysorbate 80, Cremophor EL, Emulphor EL620*
- **Anaphylaxis:** IgE-mediated - uncommon in non-clinical testing; requires susceptible individual, appropriate Ag, and time for specific antibody generation

Histamine - clinical signs that may initiate testing

Clinical signs dominated by histamine-related effects; may see:

- Cutaneous/ mucosal erythema, urticaria, pruritus, head shaking
- Peripheral edema (eg, facial / periorbital/ paws)
- ↑ Mucus secretions, diarrhea
- Blood pressure alterations, tachycardia,
- Increased plasma histamine (*dogs, monkeys, rabbits*)



- **Incidence: Dogs >> monkey, human > rodents**
- Utility of testing includes to explain observed toxicity or screen for mast cell/ basophil/ eosinophil -activating compounds/vehicles

Histamine testing - assay method; results

Histamine ELISA - adequately cross-reactivity for all common laboratory species

Sample collection intervals - correlate with clinical observations:

- Predose and serial sampling to bracket peak reaction (of IgE response) to near end of exposure
- Heparin or EDTA plasma, chilled tubes - care in handling to avoid in vitro cellular histamine release, hemolysis

Non-clinical results poorly predictive of clinical outcomes

Differences in mast cell/ basophil/ eosinophil characteristics and receptor specificities for triggering substances between species

Plasma histamine (nM) in beagle dogs administered infusion of compound X

Dose (mg/kg)	Male Dog No.	Predose	5 min	10 min	30 min	Clinical Observations				
25	1	1.9	2.0	1.6	2.3	No clinical reaction observed				
	2	10.0	3.5	4.8	3.1					
50	3	2.3	2.3	22.3	421.1	Prominent reaction (generalized cutaneous erythema, pruritis) w/ 10 minutes				
	4	4.7	2.4	2.6	5.5					
	5	2.0	3.4	2.7	9.7					
100	6	2.4	2.0	8.4	NT					
	7	2.2	3.3	6.2	91.7	Facial/pinnal erythema, head shaking w/ 10 minutes				
	8	28.2	5.4	5.0	128.2	Facial/pinnal erythema, head shaking w/ 15 minutes				

Expected normal plasma level is <10 nM.

NT indicates not tested because hemolyzed samples cannot be assayed.

Questions?



Innate or Adaptive Immunity?

Innate Immunity

Non-specific, genetically-determined immune responses with immediate maximal effect potential; without immunologic memory

Granulocytes (neutrophils, eosinophils, basophils) mast cells, monocytes/macrophages, dendritic cells, NK cells, $\gamma\delta$ T cells, endothelial and parenchymal cells

Soluble components: APRs, complement components, naturally-occurring IgM, cytokines

Adaptive Immunity

Antigen-specific responses, non-genetically-determined, induced - with lag time for maximal response; based on immunological memory

B-cells, and CD4+, CD8+ T cells, long-lived APCs - distinct tissue distribution

Soluble components: acquired immunoglobulins (IgG, IgA, IgE, IgM), cytokines

Serum Immunoglobulin (Ig) class analysis (IgG, IgM, IgA, IgE)

Quantifying a serum Ig class may be of value to evaluate:

Total serum hyper-/ hypo- globulinemia

B- or T-helper cell functional effects

Selective lymphoproliferative or autoimmune-like responses

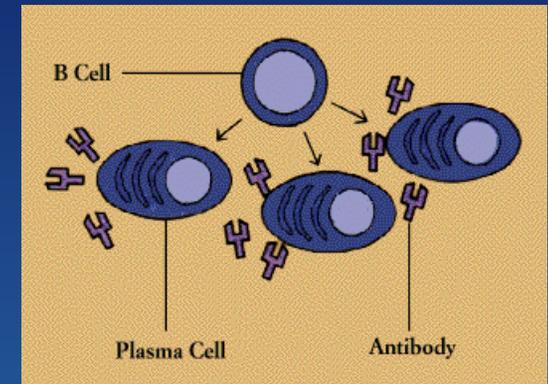
Reflects general responses of lymphoid cell types involved and Ag exposures in adult animals

➤ **IgG** = 70-75% of total serum Ig concentration

▪ *Dependent upon B- cells, & T-helper cell co-stimulation and cytokine production*

➤ **IgM** = ~10% of total serum Ig

▪ *Partly independent of T-helper cell responses*



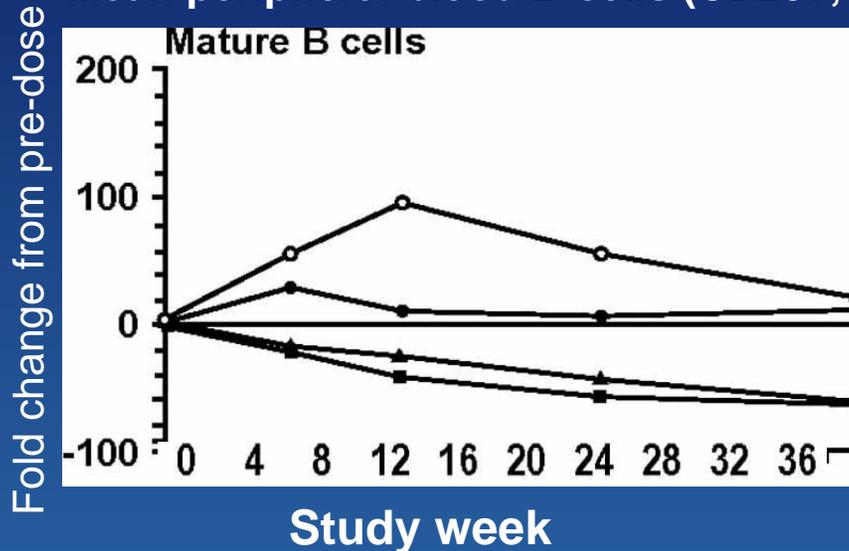
Ig class analysis - considerations in testing & interpretation

- Non-specific as to humoral response to specific antigens (and may not correlate with results of functional testing)
- Not a measure of humoral immunocompetence, or drug-associated immunogenicity
- Species-specific assays; multiplex methods available for quantifying all classes in same sample
 - Allow adequate time for a response/ inter-interval change
- Some species functional differences in IgG subclasses - may be important for clinical relevance:

<u>Species</u>	<u>IgG subclasses</u>
Human	IgG1, IgG2, IgG3, IgG4
Rhesus/Cyno	IgG1, IgG2, IgG4
Dog	IgG1, IgG2
Rat	IgG1, IgG2a, IgG2b, IgGc, IgG3, IgG4

Serum IgG & IgM in monkeys given B-cell maturation/proliferation inhibitor

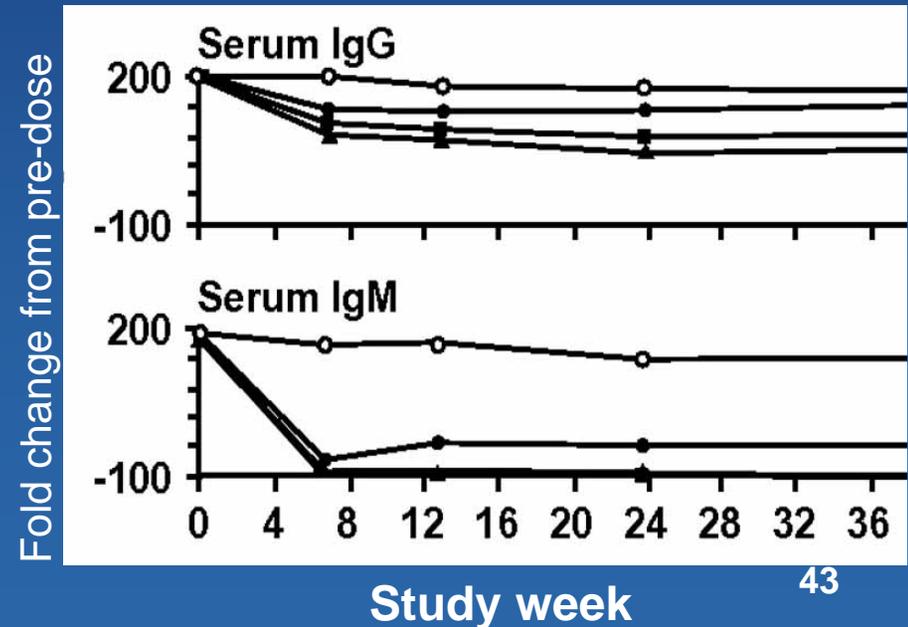
Mean peripheral blood B-cells (CD20+, IgD+):



- = 0 mg/kg (Vehicle)
- = 0.4 mg/kg
- = 2 mg/kg
- ▲ = 10 mg/kg

Cynomolgus monkeys SC dosed 2x/week

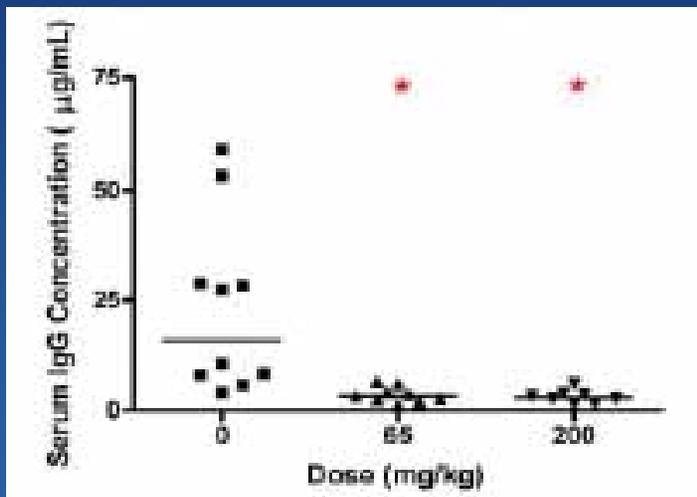
Mean serum Ig class concentration:



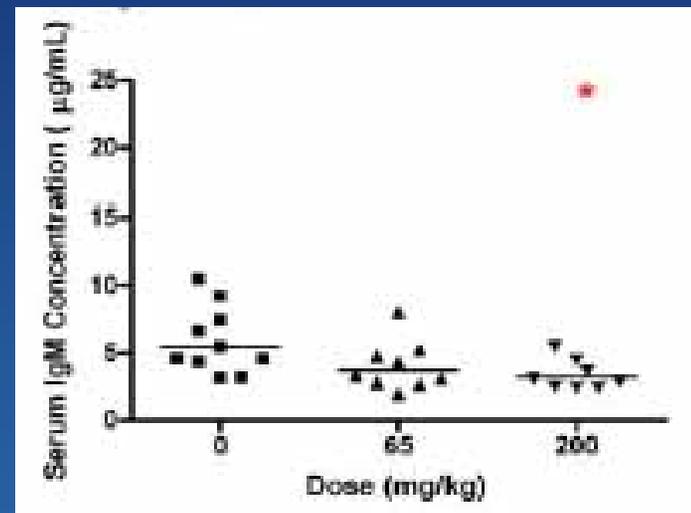
Serum IgG & IgM in rats given an inhibitor of CD4⁺ T-cell activation

Male rats dosed Q3d at 0, 65 or 200 mg/kg

Serum total **IgG** at week 4



Serum total **IgM** at week 4



Peripheral blood: ↑ CD3⁺, CD4⁺ T-cells;
No effect on total B-cells (CD3⁻CD45RA⁺)



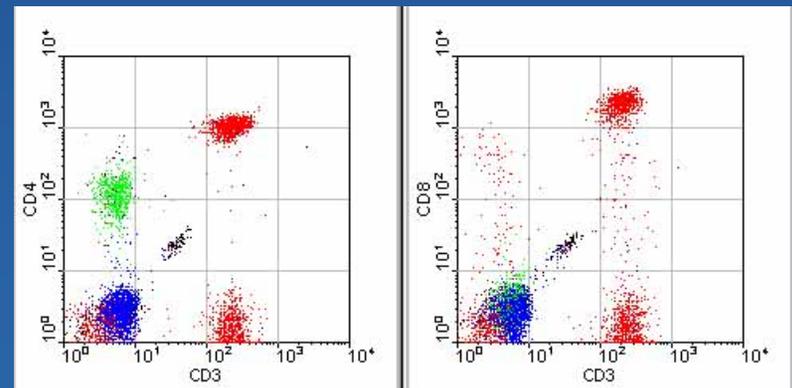
aargh, What's Next...!?

Peripheral blood lymphocyte phenotyping

Readily included on study - standard hematology samples, minimum added volume requirements

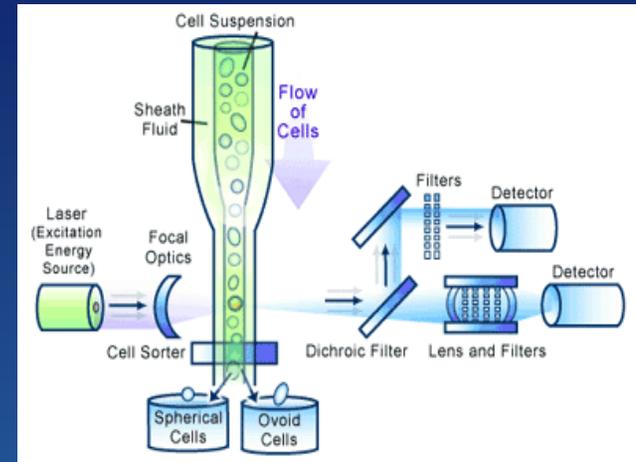
- **Most commonly used to enumerate total B-, & T- (CD4⁺, CD8⁺) lymphocytes**
 - ~ more comprehensive screen of lymphoid subtype #s than tissue (spleen, thymus, lymph node) analysis
 - May aid interpretation of anatomical pathology findings and/or functional tests

- **Also used to quantitate (eg):**
 - NK cells
 - Membrane receptors, activation markers
 - Membrane-bound Ig, maturation stages
 - Intracellular proteins



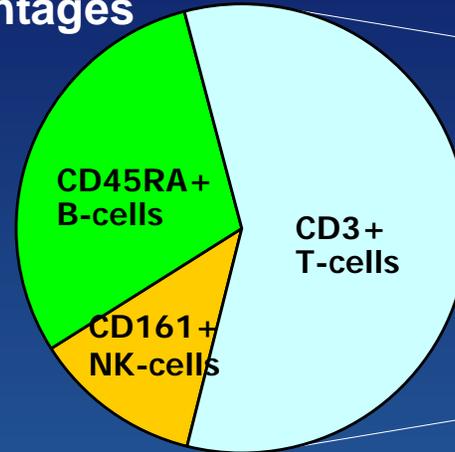
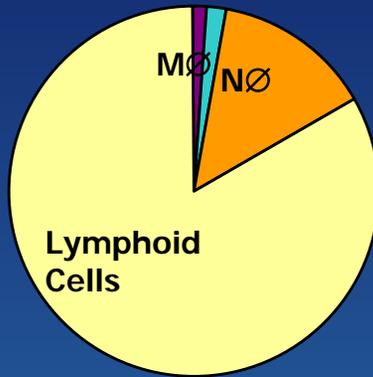
Lymphocyte phenotyping -considerations for testing and interpretation

- Flow cytometers are technically complex to operate
- Assays ~ difficult to GLP validate
- Samples must be processed fresh - generally within ≤ 24 hrs
- Sample collection, processing - critically affect results
- As with leukocyte differential:
 - Dynamic values (ensure adequate animal #s/group)
 - 2° influences - catecholamines, GC, inflammation, etc.
 - Major species differences in subtype population distribution



Lymphocyte/ NK cell phenotyping - species differences

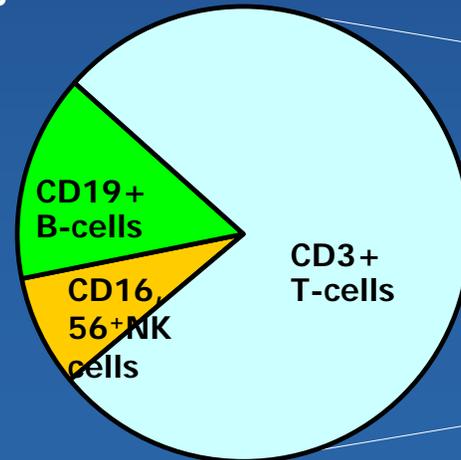
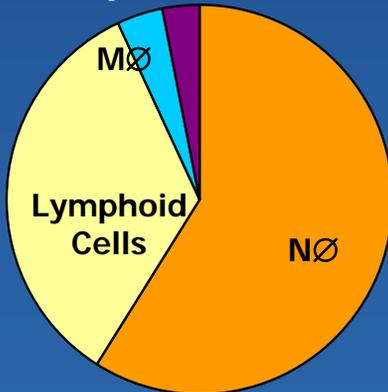
Rat Peripheral Blood Leukocyte Percentages



CD3+CD4+
35-40%

CD3+CD8+
20-25%

Human Peripheral Blood Leukocyte Percentages

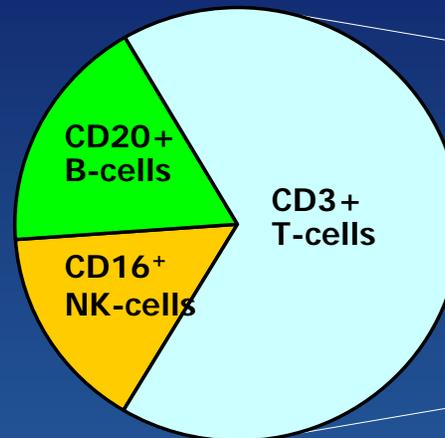
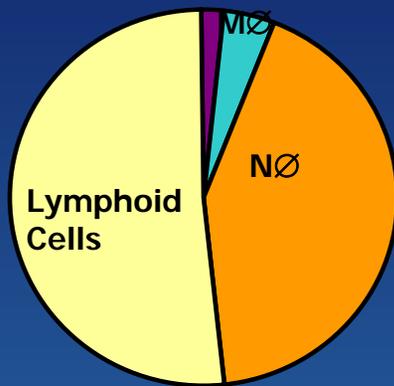


CD3+CD4+
40-50%

CD3+CD8+
20-30%

Leukocyte/Lymphocyte/ NK cell Phenotyping - species differences

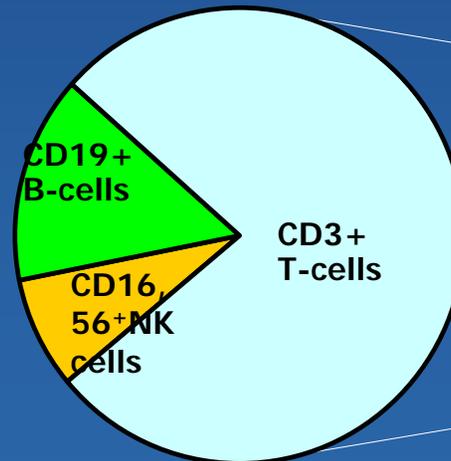
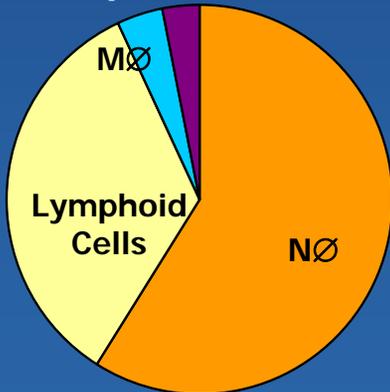
Monkey Peripheral Blood Leukocyte Percentages



CD3+CD4+
25-35%

CD3+CD8+
20-30%

Human Peripheral Blood Leukocyte Percentages

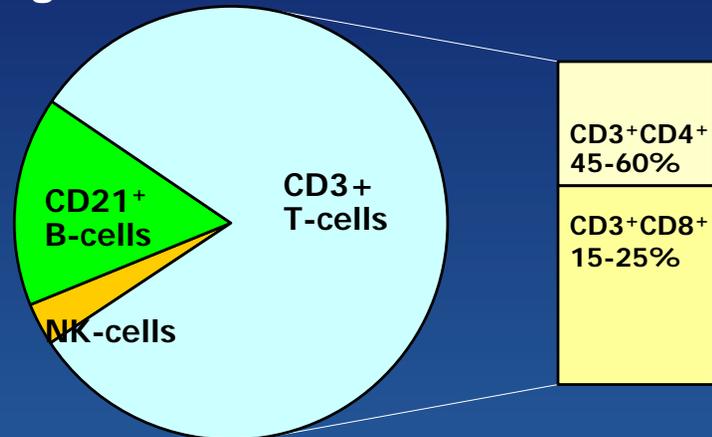
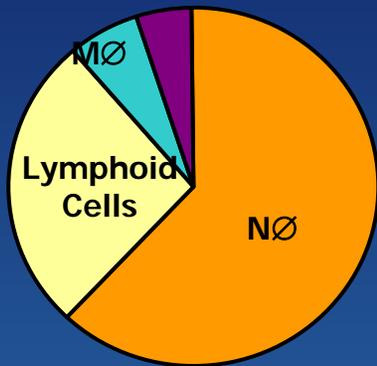


CD3+CD4+
40-50%

CD3+CD8+
20-30%

Leukocyte/Lymphocyte/ NK cell Phenotyping - species differences

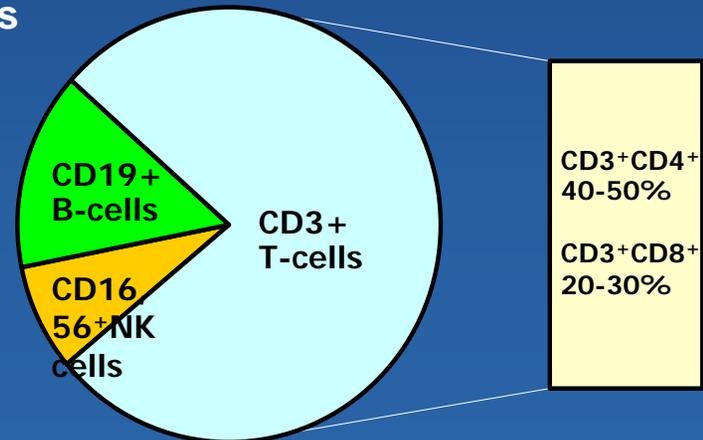
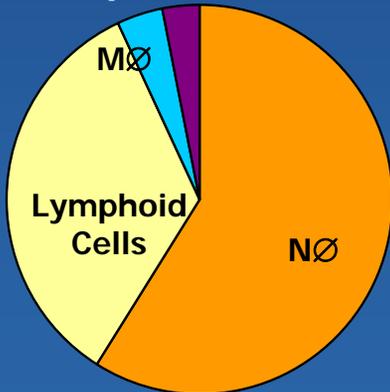
Dog Peripheral Blood Leukocyte Percentages



CD3+CD4+
45-60%

CD3+CD8+
15-25%

Human Peripheral Blood Leukocyte Percentages



CD3+CD4+
40-50%

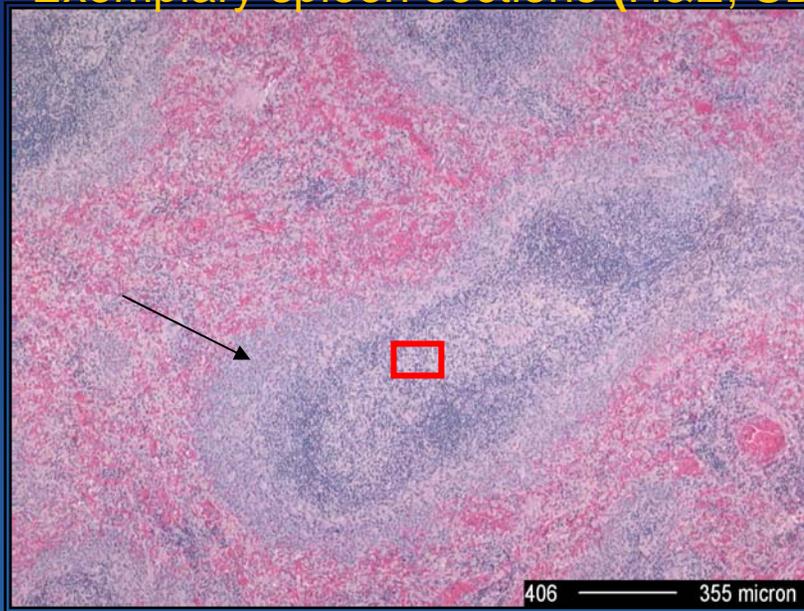
CD3+CD8+
20-30%

Leukocyte/Lymphocyte/ NK cell Phenotyping - testing considerations

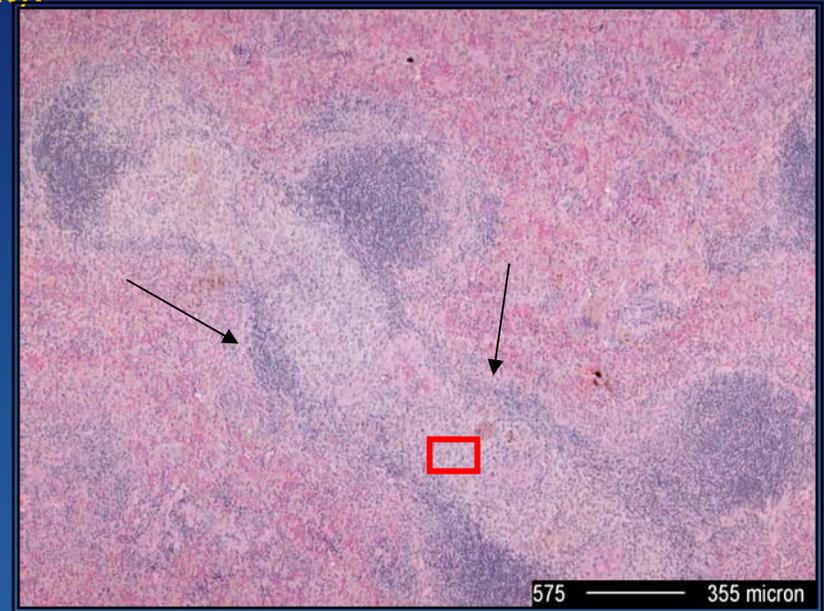
- Which species is more relevant?
 - Differences among all species in cell subtype distribution in blood, and some activation/ other membrane markers
 - Species differences in availability of reagents (labeled Abs)
 - Human reagents often cross-react with monkey
 - No non-human species is ideal model for humans
- When to analyze?
 - Case-by-case approach to investigate, eg:
 - The cause of an unexplained ↓ or ↑ in total blood lymphocyte counts
 - The cause of, or peripheral blood links with a histologic change
 - Utility as potential translational biomarker(s)
 - Efficacy of an immunomodulatory compound

Case example: histologic finding in the spleen of rats

Exemplary spleen sections (H&E, SD rat):



(Control - male)



(Group 3 -male)

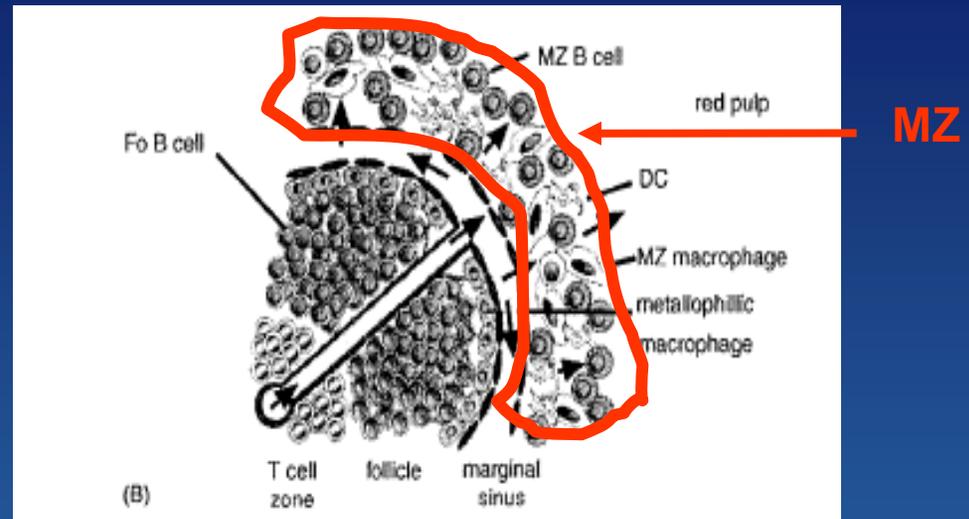
Decreased Marginal zone (MZ) thickness, lymphocyte depletion - PALS (& RE cell hyperplasia/prominence) - dose-related in all test article-administered groups compared to controls.

Regarding histologic finding in the spleen: MZ cell functions

Marginal Zone B-cells & mØ have a ~1° role in T-cell Independent Antibody Response

(ie. to certain blood borne antigens - especially bacterial PS, repetitive GPs)

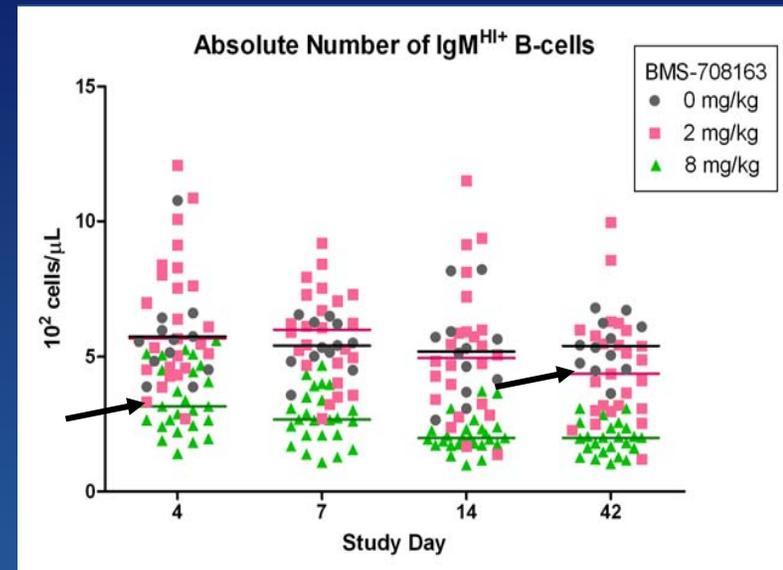
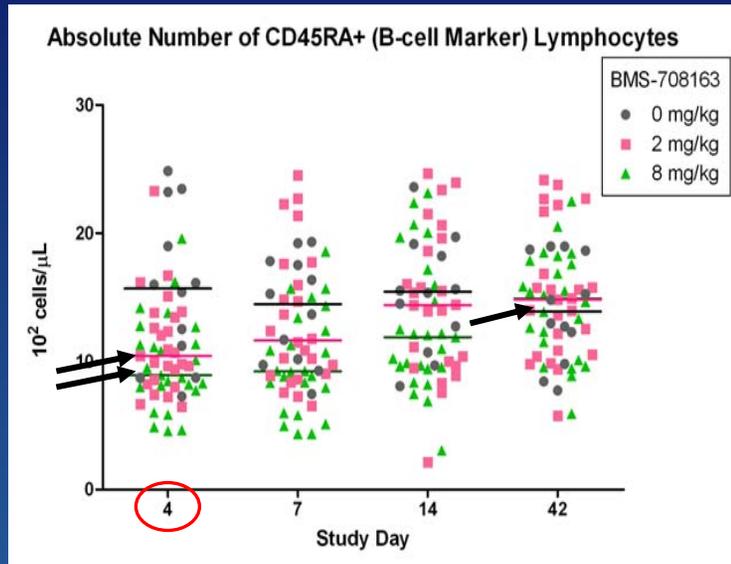
MZ B-cells are principally IgM^{hi}



➤ Follow-up extended study with satellite groups for peripheral blood & splenic lymphocyte subtype counts:

- Counts of total (CD45RA +) & IgM^{hi} B-cells; total, CD4⁺ & CD8⁺ T-cells, & hematology - days 4, 7, 14, 42
- Histopathology - weeks 2 & 6

Peripheral blood lymphocyte phenotyping for B-cell (CD3-/CD45RA⁺) & IgM^{hi} expression



N=12

- ❖ Day 4 - **Total B-cell counts** are ↓ in a dose dependent manner (less pronounced difference from controls at subsequent intervals)
- ❖ Day 4 - **B-cell IgM^{hi} cells** ↓ at high dose (8 mg/kg). At Day 42 - ↓ at low dose (2 mg/kg) also.
- ❖ Total lymphocyte counts, and CD4+ T-cell counts - slight ↓ at high dose - wk 2 & 6
- ❖ **Clinical screening for peripheral blood IgM^{hi} B cell, and CD4+ T cell counts, and a rat host resistance study (*Strep. pneumoniae*) were initiated**

Peripheral blood lymphocyte phenotyping - final thoughts

- Findings can be used to establish target cell population and/or determine next steps for functional testing, set clinical biomarkers, mechanism of effect
- Effects on blood lymphocyte subtype counts alone do not determine immunosuppression or immunostimulation, or necessarily a 1° effect
- Recommended if same-species functional assays ~or tissue lymphocyte phenotyping planned
- Keep in mind that there are major species-, age-, and sex- related differences in lymphocyte subpopulations - \pm responses
- Always consider the potential for artifact due to sample stability/handling
- Routine lymphocyte phenotyping is generally unrewarding

Immunotoxicologic Pathology: clinical biomarkers in non-clinical testing - conclusions

- Evaluate changes in clinical pathology immune-system-related data in conjunction with all other study results
- Interpret clinical pathology findings collectively and look for common patterns
- Consider whether changes are secondary or compensatory rather than specific/direct
- Consider clinical relevance
 - Species differences in endpoints evaluated, and responses in general (eg, hypersensitivity)
 - Exposure multiples & risk/ benefit ratio
 - Translational biomarkers for monitoring
- Follow-up work is best driven by the specific findings, and to address specific questions on the immune response observed

Questions?

