Immune side effects of biologicals and nanomedicines:

unsolved issues in bio- and nanosimilar development

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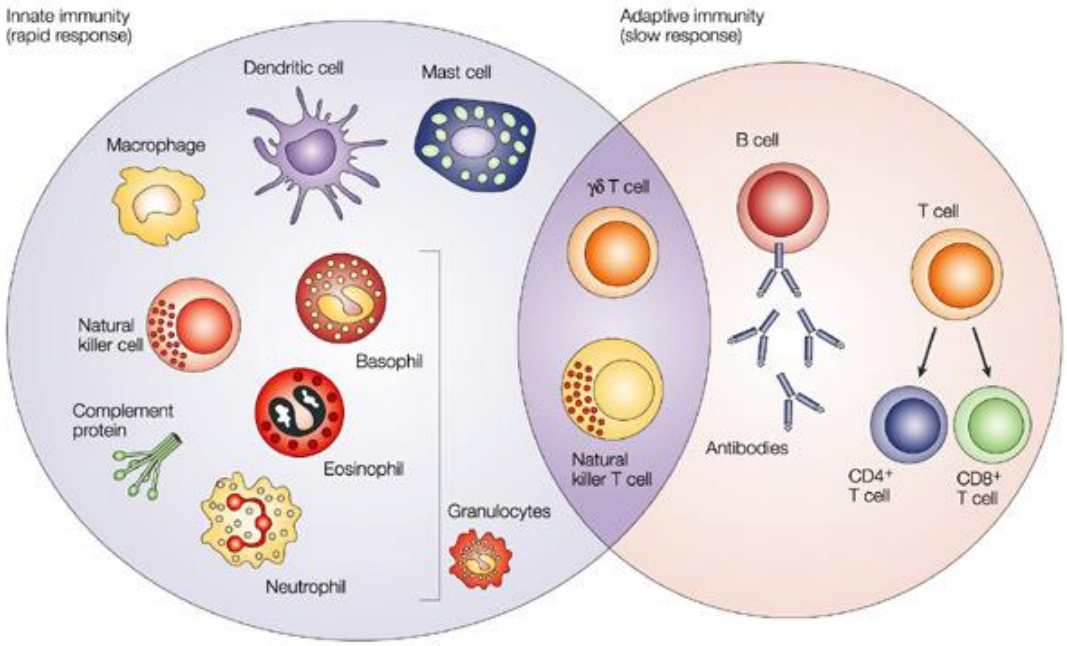
Outline

- **Immune system and nanomedicines** *(reminder/primer, update)*
- **Adverse interactions of biologicals and nanomedicines with the immune system**
  - **acut hypersensitivity reactions**
    - **Terminology**
    - **Basic info**
    - **Role of complement (C)**
  - **immunogenicity**
- **Regulatory „reaction” to adverse immune effects**
- **Experiments suggesting the use of pigs for assessing immune reactivity and immunogenicity**

The immune system

System of organs, dispersed cells and bound or soluble molecules that protect the body from external and internal harm in a highly organized fashion

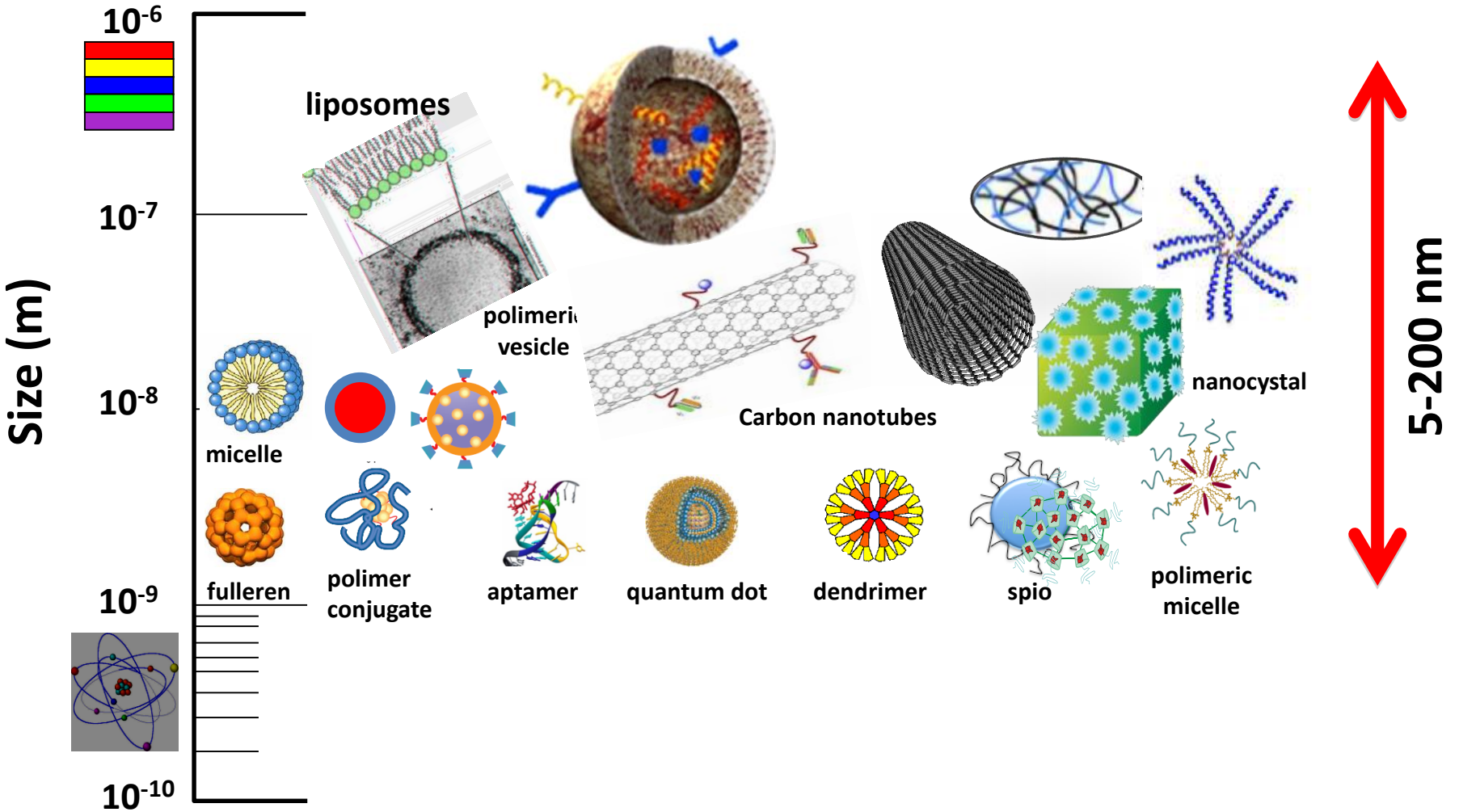
- **Organs**
  - **Primary**
    - **thymus, spleen,**
  - **Secondary**
    - **lymph nodes**
- **Cells**
  - **Specific (adaptive)**
  - **Nonspecific (innate)**
- **Molecules**
  - **Specific (adaptive)**
  - **Nonspecific (innate)**



Basic features of nanomedicines

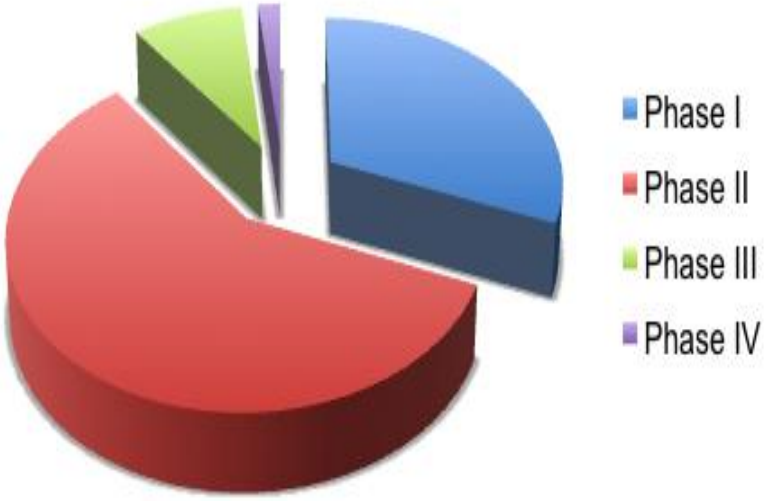
- Size in the 10-1000 nm range (macromolecules and cell organs)
  - Nano-paradox (larger than conventional drugs and biologicals)
- Multicompartment/Composite structure
  - carrier (nanocarrier/nanovehicle) for controlled release and targeting
  - payload for therapy, imaging, sensing
- Multifunctionality
  - Targeting
    - Passive, active
  - Imaging (contrast agents)
  - Release control (depot)

Drug carrier nanosystems



Nanoplatforms in clincical trials

Platfom	Cancer	Global
Liposome	443	590
Polymer	32	204
Gold	171	1110
Silver	104	379



www.clinicaltrials.gov

Products

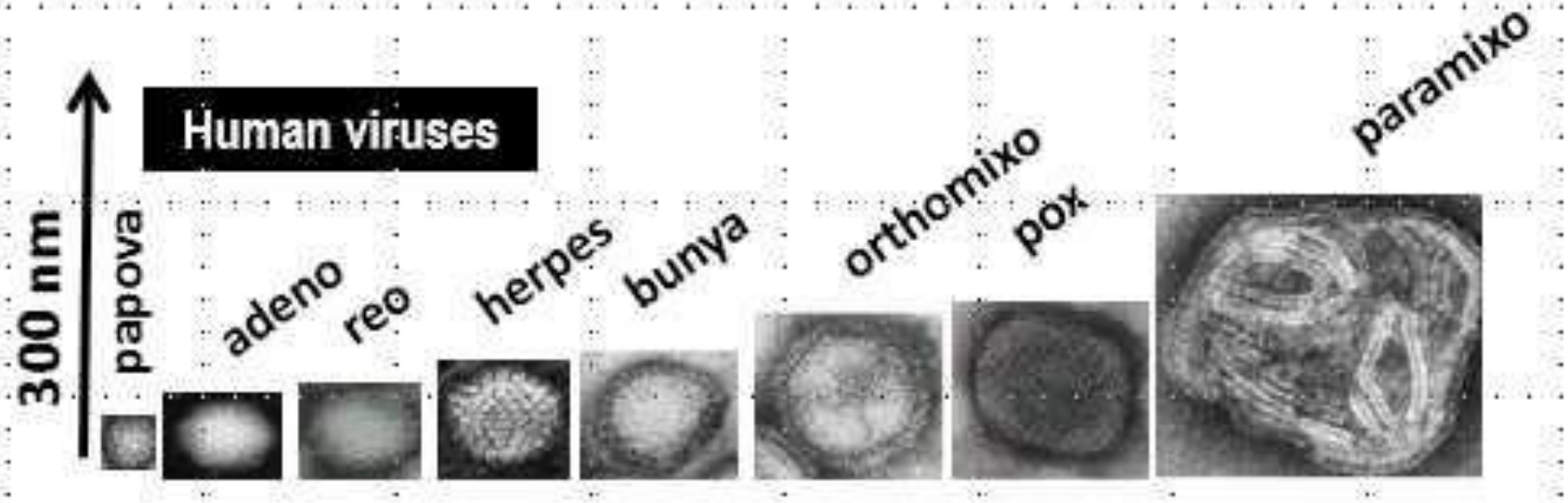
Sector	Number of products	Companies
Drug delivery	23	113
Biomaterials	9	32
In vivo imaging	3	13
In vitro diagnostics	2	35
Active implants	1	7
Misc	0	7
Total	38	207

The emerging nanomedicine landscape
Volker Wagner, Anwyn Dullaart, Anne-Katrin Bock & Axel Zweck
NATURE BIOTECHNOLOGY VOLUME 24

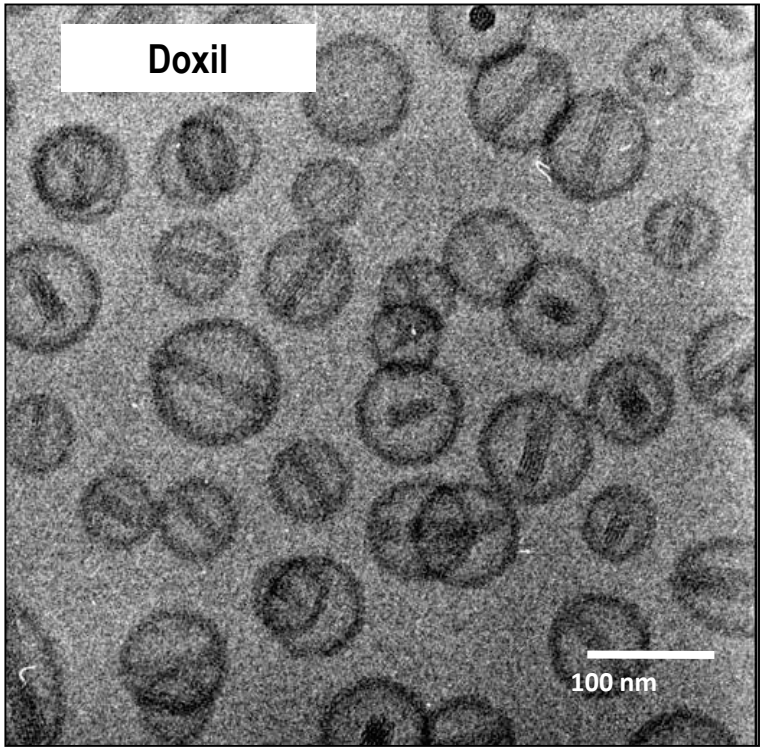
Further statistics on Europen nanomedicine activity

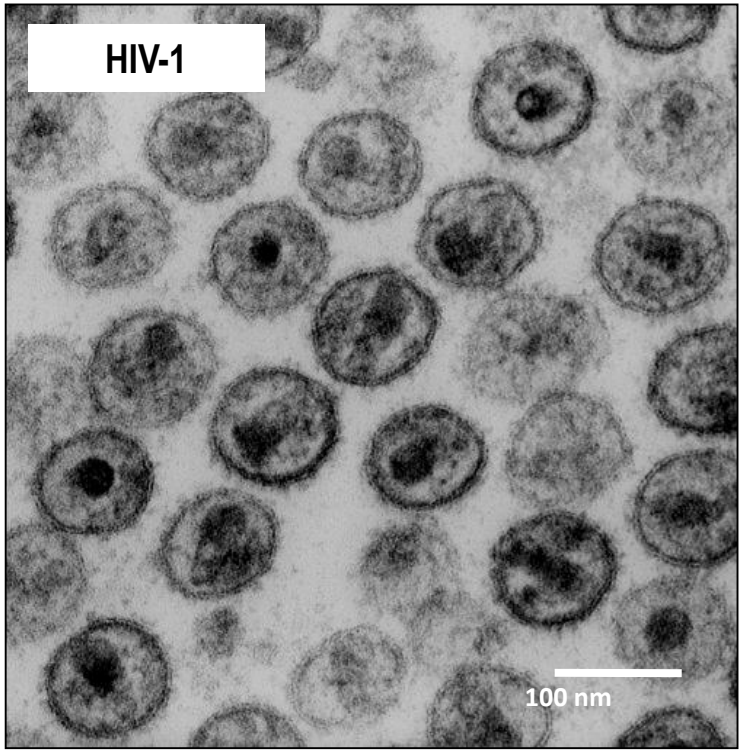
- 86 projects with budget: 446 M EUR
  - 1700 institutions
  - 400 industrial partners
  - Horizon 2020 100 M EUR already invested
- EU NCL network established
- Yearly Clinam conference with >500 participants >40 countries, >10 years
- 10,000 publications until 2016
- Poduct revenue:
  - present 151 M USD
  - 2023: 392 M USD

Why the immune system comes to play in nanomedicine?

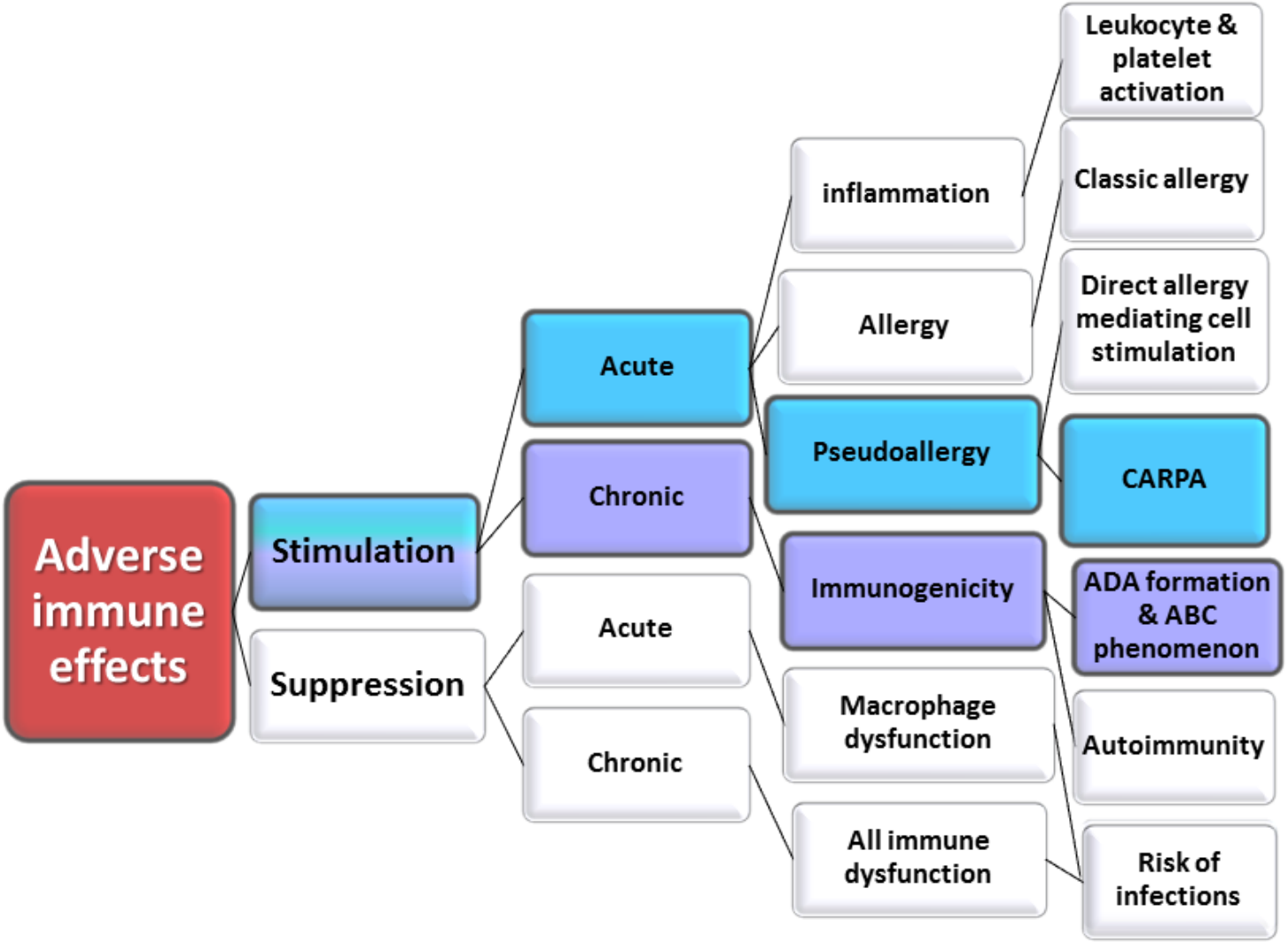


Morphological similarity between liposomes and pathogenic human viruses





The adverse immune effects of nanomedicines and biologicals



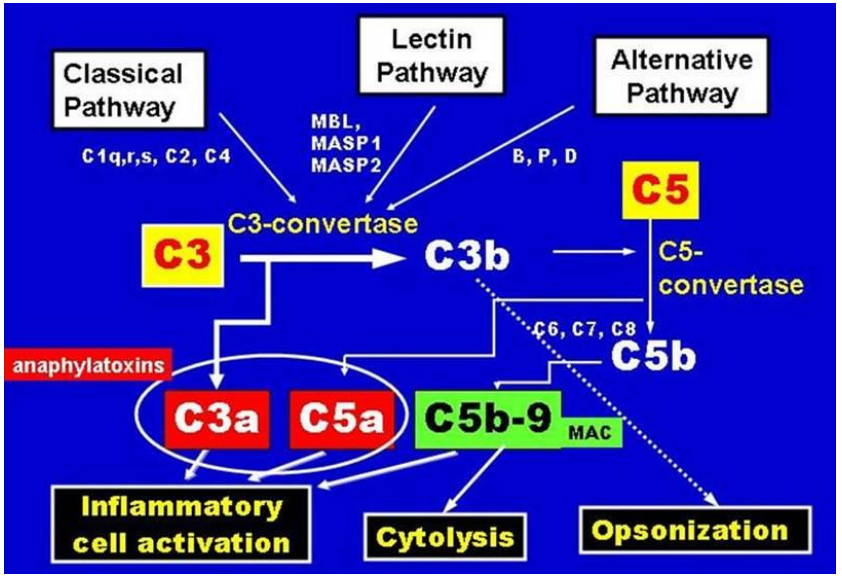
Redundant and confusing terminology of hypersensitivity reactions to I.V. drugs

- **Infusion reaction**
- **Idiosyncratic reaction**
- **Anaphylaxis**
- **Pseudoallergy**
- **Non-allergic hypersensitivity**
- **Non-immune hypersensitivity**
- **Complement-activation-related pseudoallergy (CARPA)**
  - ***Causal/functional term***

The CARPA concept

A large fraction of acute hypersensitivity (allergic) reactions to I.V. drugs is caused by complement (C) activation, or at least C activation is a key contributor to these reactions.

Many state-of-art anticancer and other nanomedicines and therapeutic antibodies have heightened risk to acivate C, and, hence, cause CARPA



- first treatment (no prior exposure)
- milder or absent upon re-exposure
- spontaneous resolution
- pulmonary infiltration
- high reaction rate (2-10%) or higher

Incidence of pseudoallergy

- ≈ 2 million adverse drug reactions with 106,000 fatalities per year represents the 4th to 6th leading cause of death in the USA.
- ≈ 25 % of all adverse drug reactions is allergic in nature.
  Lazarou et al., *JAMA*, 279;1200, 1998

- ≈ 80 % of all allergic drug reactions is not true allergy.
  Demoly et al., *Allergy*, 54;500, 1999

  400,000 pseudoallergic reactions USA/year
- ≈ 0.1% of reactions is fatal
  - **400 fatal reactions USA/year**
  - **fatal reactions in the order of 1000s worldwide/year (orphan disease)**

- 10,000 anaphylactic reactions/year, mainly food and insect bite (750 deadly (7.5%)

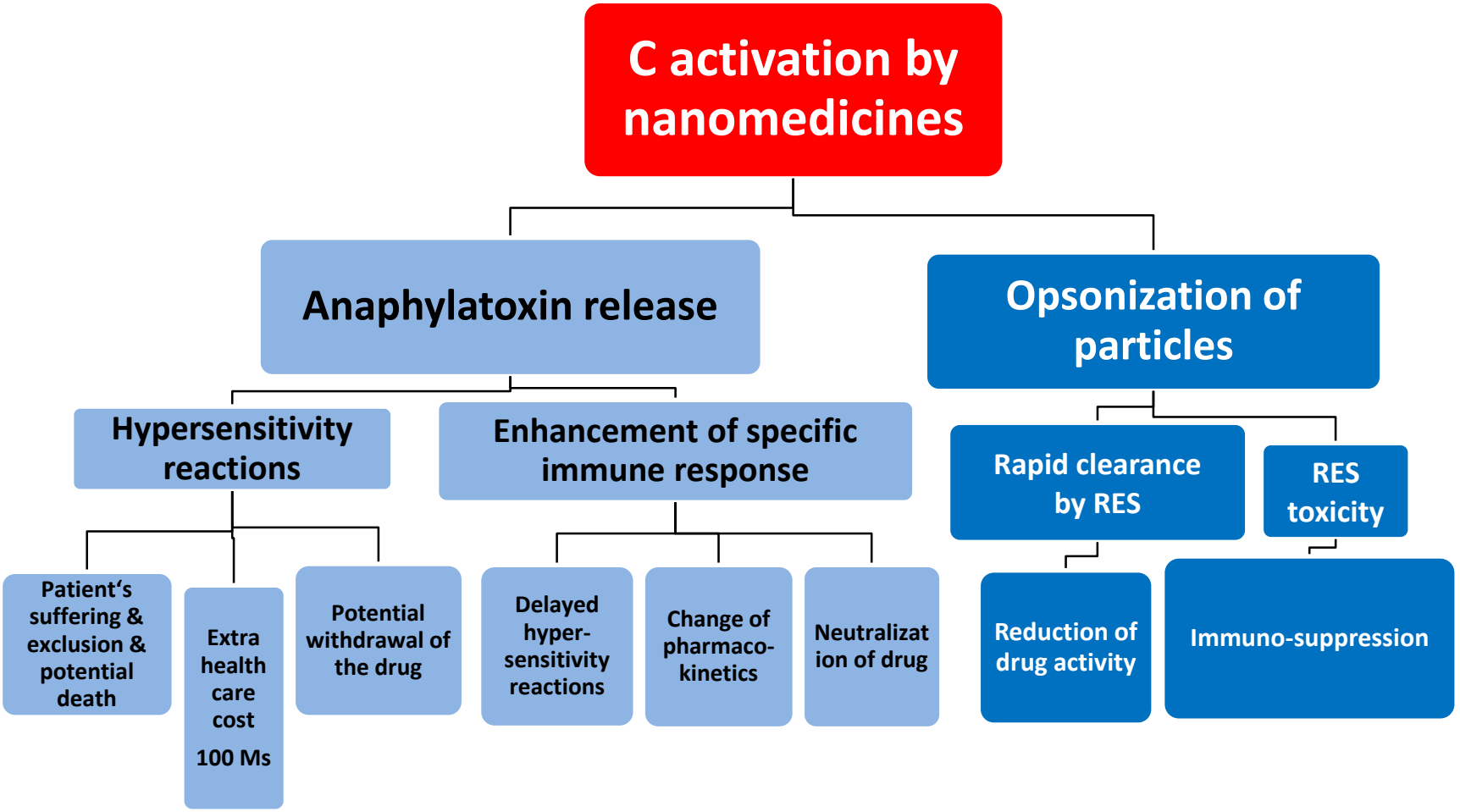
Drugs Causing Pseudoallergy (Infusion reactions)

Liposomal drugs and diagnostics	Micellar drug formulations	Radio and ultrasound contrast agents	Antibody-based Therapeutics & diagnostics	Enzymes Proteins Peptides	Miscellaneous other
Doxyl (Caelix) Ambisome Amphocyl Myocet DaunoXome Tc^{99}-HINIC-PEG	Taxol Taxotere Cyclosporine Etoposide poloxamers	Diatrizoate Iodixanol Iohexol Iopamidol Iopromide Iothalamate Ioversol Ioxaglate Ioxilan SonoVue Magnevist	Avastin Enbrel Herceptin Humira Raptiva Synagis Xolair Compath Erbitux Mylotarg Remicade Rituxan Vectibix Tysabri	Avonex Actimmune Abbokinase Aldurazyme Activase Zevalin Neupogen Neulasta Fasturtec Plenaxis	Cancidas Copaxone Orencia Eloxatin Salicilates

Organ manifestations of CARPA

Cardio-vascular	Broncho-pulmonary	Hemato-logical	Muco-cutaneous	Gastro-intestinal	Neuro-psycho-somatic	Systemic
angioedema	apnea	leukopenia	cyanosis	nausea	back pain	chills
arrhythmia	bronchospasm	granulopenia	erythema	vomiting	chest pain	diaphoresis
cardiogenic shock	coughing	rebound leukocytosis	flushing	metallic taste	chest tightness	fever
hypertension	dyspnea	rebound granulocytosis	rash	diarrhea	headache	sweating
hypotension	hyper-ventilation	thrombocyto-penia	rhinitis	cramping	feeling of imminent death	wheezing
hypoxia	laryngospasm	lymphopenia	swelling	bloating	fright	rigors
myocardial infarction	stridor		urticaria		panic	feeling of warmth
tachycardia	respiratory distress		nasal congestion		rigors	loss of consciousness
ventricular fibrillation	shortness of breath		pruritus		anxiety	*death*
edema	sneezing		tearing		confusion	
syncope	hoarseness		conjunctival erythema		dizziness	

Clinical significance of CARPA: dual harm

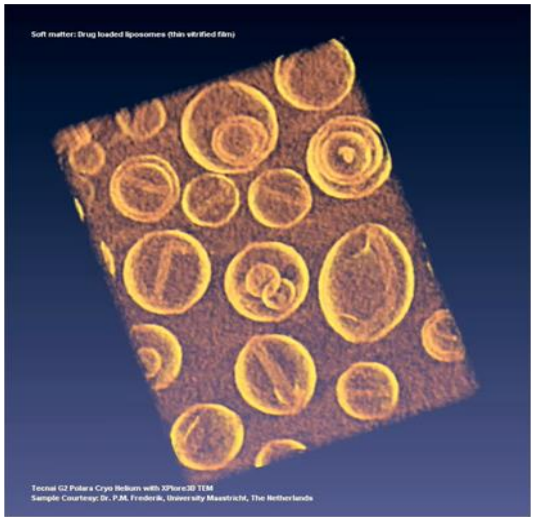


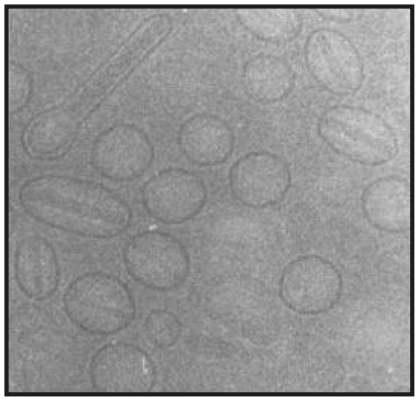
Hypersensitivity reactions to marketed liposomal drugs

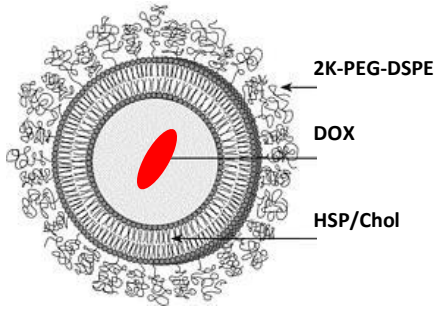
Brand name (manufacturer)	Active ingredient	Indication	Type of particle (Size)	Symptoms
Doxil, Caelyx (Johnson & Johnson)	doxorubicin	ovarian cancer, Kaposi sarcoma, myeloma multiplex	liposomes (80-100 nm)	flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, hypotension
Myocet (Elan)				flushing, dyspnea, fever, facial swelling, headache, back pain, chills, tightness in the chest and throat, hypotension
Abelcet (Elan, Enzon)	amphotericin B	fungal infections	solid microparticles (1.6-11 mm)	shortness of breath, change in blood pressure
Ambisome (Gilead, Fujisawa)			liposomes (45-80 nm)	chills, rigors, fever, nausea, vomiting, cardiorespiratory events
Amphotec, Amphocyl (Elan)			disk shape solid nanoparticles (115 nm)	hypotension, tachycardia, bronchospasm, dyspnea, hypoxia, hyperventilation
DaunoXome (Gilead)	daunorubicin	Kaposi sarcoma	liposomes (45 nm)	back pain, flushing, chest tightness
Visudyne (Novartis)	verteporfin	age-related macular degeneration	multilamellar liposomes (multimicron)	chest pain, syncope, sweating, dizziness, rash, dyspnea, flushing, changes in blood pressure and heart rate, back pain

Szebeni J. Haemocompatibility testing for nanomedicines and biologicals: Predictive assays for complement mediated infusion reactions. Eur. J. Nanomedicine, 4 (1), 33-53

Doxil (liposomal doxorubicin)







„*WARNING:* Acute infusion-related reactions, sometimes reversible upon terminating or slowing infusion, occurred in up to 10% of patients. Serious and sometimes fatal allergic/anaphylactoid-like infusion reactions have been reported. Medications/ emergency equipment to treat such reactions should be available for immediate use …" Source: www.doxil .com

Hypersensitivity reactions to some marketed monoclonal antibodies

Szebeni J, Eur. J. Nanomedicine, 4 (1), 33-53, 2012

Brand name (manufacturer)	mAb, type (target antigen)	Indication	Incidence	Symptoms
Anti-cancer use				
Avastin (Genentech/Roche)	bevacizumab, recombinant humanized IgG_1 (VEGF-A)	Combination chemotherapy of metastatic colon, lung, kidney cancer and glioblastoma	< 3%, severe: 0.2%	chest pain, diaphoresis, headache, hypertension, neurologic signs and symptoms, oxygen desaturation, rigors, wheezing
Campath (Genzyme)	alemtuzumab)–IH, recombinant, humanized IgG_1k (CD52 on T and B cells)	B cell chronic lymphocytic leukemia (B-CLL)	4-7%	bronchospasm, chills, dyspnea, emesis, fever, hypotension, nausea, pyrexia, rash, rigors, tachycardia, urticaria
Erbitux (Bristol-Myers Squibb, Eli Lilly)	cetuximab, chimeric IgG_1k (human EGFR)	metastatic colorectal cancer, head and neck cancer, squamous cell carcinomas	< 3%, fatal <0.1%	anaphylaxis, angioedema, bronchospasm, cardiac arrest, chills, dizziness, dyspnea, fever, hoarseness, hypotension, pruritus, rash, rigor, stridor, urticaria, wheezing
Herceptin (Genentech)	trastuzumab , humanized IgG_1k (human EGFR receptor 2, HER2/neu / erbB2)	metastatic breast and gastric cancer	<1%	asthenia, bronchospasm, chills, death within hours, dizziness, dyspnea, further pulmonary complications, headache, hypotension, hypoxia, nausea, pain, rash, severe hypotension, vomiting
Mylotarg (Pfizer Inc./Wyeth Pharmaceuticals)	gemtuzumab ozogamicin. recombinant humanized IgG_4k (CD33 on hematopoetic cells)	CD33 positive acute myeloid leukemia in first relapse	<8%	acute respiratory distress syndrome, anaphylaxis, dyspnea, fatal anaphylaxis, hypotension, pulmonary edema
Vectibix (Amgen)	panitumumab, recombinant humanized IgG_2k (human EGFR)	KRAS+ metastatic colorectal carcinoma	1-4%	anaphylactic reaction, bronchospasm, chills, fever, hypotension
Rituxan (Genentech)	Rituximab, chimeric IgG_1k (CD20 on B cells)	B cell leukemias, rheumatoid arthritis and non-Hodgkin's B-cell lymphoma	**>80% severe: <10%**	ARDS, bronchospasm, cardiogenic shock, flushing, hypotension, hypoxia, itching, myocardial infarction, pain (at the site of the tumor), pulmonary infiltrates, runny nose, swelling of the tongue or throat, ventricular fibrillation, vomiting
Anti-inflammatory use				
Remicade (Janssen Biotech. Inc.)	Infliximab, chimeric IgG_1k (TNFa)	Crohn's disease, rheumatoid arthritis, spondylitis ankylopoetica, arthritis psoriatica, ulcerative colitis	**18%**	Bronchospasm, laryngeal edema, pharyngeal edema, dyspnea, hypotension, urticaria, serum sickness-like reactions
Xolair (Genentech)	Omalizumab, recombinant, humanized IgG_4 (IgE)	atopia, asthma	39% Severe: 0.2%	Anaphylaxis, bronchospasm, hypotension, syncope, urticaria, and/or angioedema of the throat or tongue, delayed anaphylaxis (with onset two to 24 hours or even longer) beyond 1 year after beginning regularly administered treatment

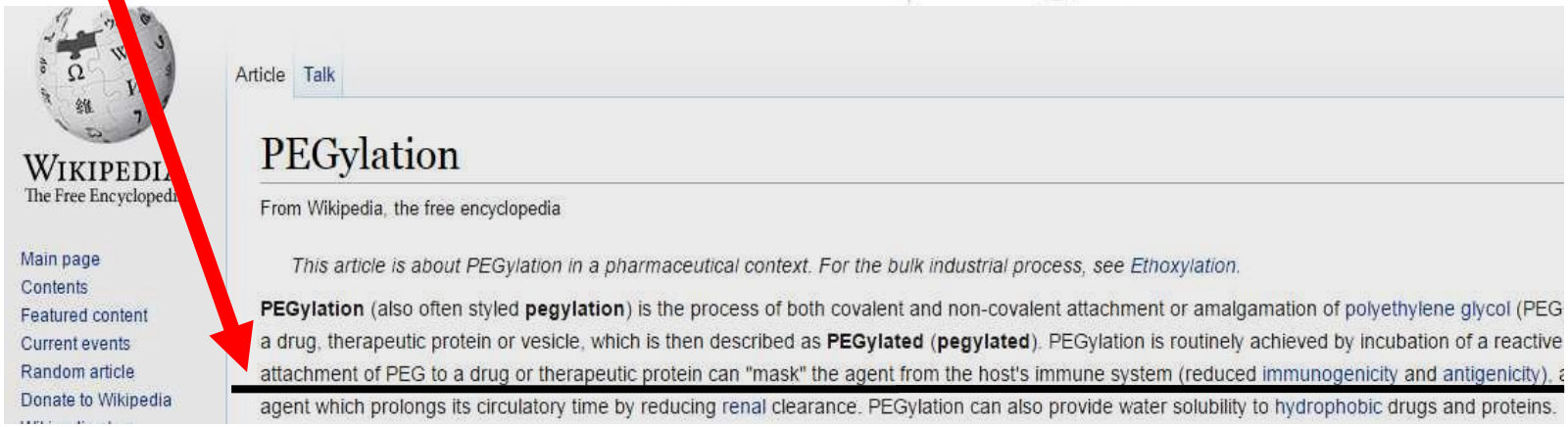
CARPA caused by PEGylated liposomes and proteins: The PEG paradox

Inreased circulation time

Decreased interaction with plasma proteins

Immune reactivity -> Hypersensitivity reactions (HSRs), ***CARPA***

Immunogenicity ***ADA formation***



Article Talk

PEGylation

From Wikipedia, the free encyclopedia

This article is about PEGylation in a pharmaceutical context. For the bulk industrial process, see Ethoxylation.

PEGylation (also often styled **pegylation**) is the process of both covalent and non-covalent attachment or amalgamation of polyethylene glycol (PEG a drug, therapeutic protein or vesicle, which is then described as **PEGylated** (**pegylated**). PEGylation is routinely achieved by incubation of a reactive attachment of PEG to a drug or therapeutic protein can "mask" the agent from the host's immune system (reduced immunogenicity and antigenicity), a agent which prolongs its circulatory time by reducing renal clearance. PEGylation can also provide water solubility to hydrophobic drugs and proteins.

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Hypersensitivity reactions to marketed PEGylated proteins

Brand name (manufacturer)	Conjugate	Indication	Symptoms
Adagen (Enzon)	PEG-adenosine deaminase	Immuno-deficiency	Acute respiratory distress syndrome, anaphylaxis, angioedema, arthralgia, bronchospasm, chills, dyspnea, edema, erythema, fever, flushing, hives, hypotension, induration, injection site reactions, lip edema, pain, skin rash, swelling, tenderness, urticaria
Neulasta (Amgen)	PEG-Filgrastim (G-CSF)	febrile neutropenia	
Oncaspar, Pegaspargase (Enzon, Rhône-Poulenc Rorer)	PEG-L-asparaginase	lympoblastic leukemia	

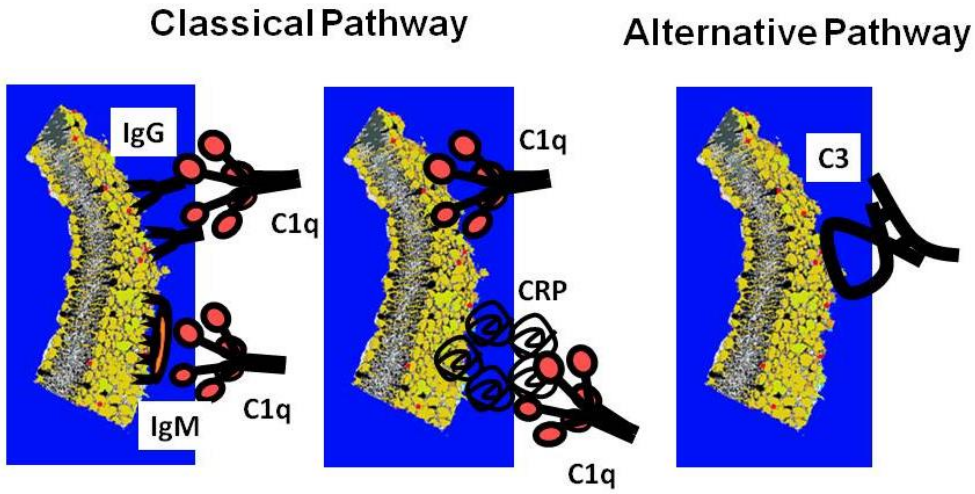
Szebeni J. Haemocompatibility testing for nanomedicines and biologicals: Predictive assays for complement mediated infusion reactions. Eur. J. Nanomedicine, 4 (1), 33-53

Significance of CARPA for the pharmaceutical industry

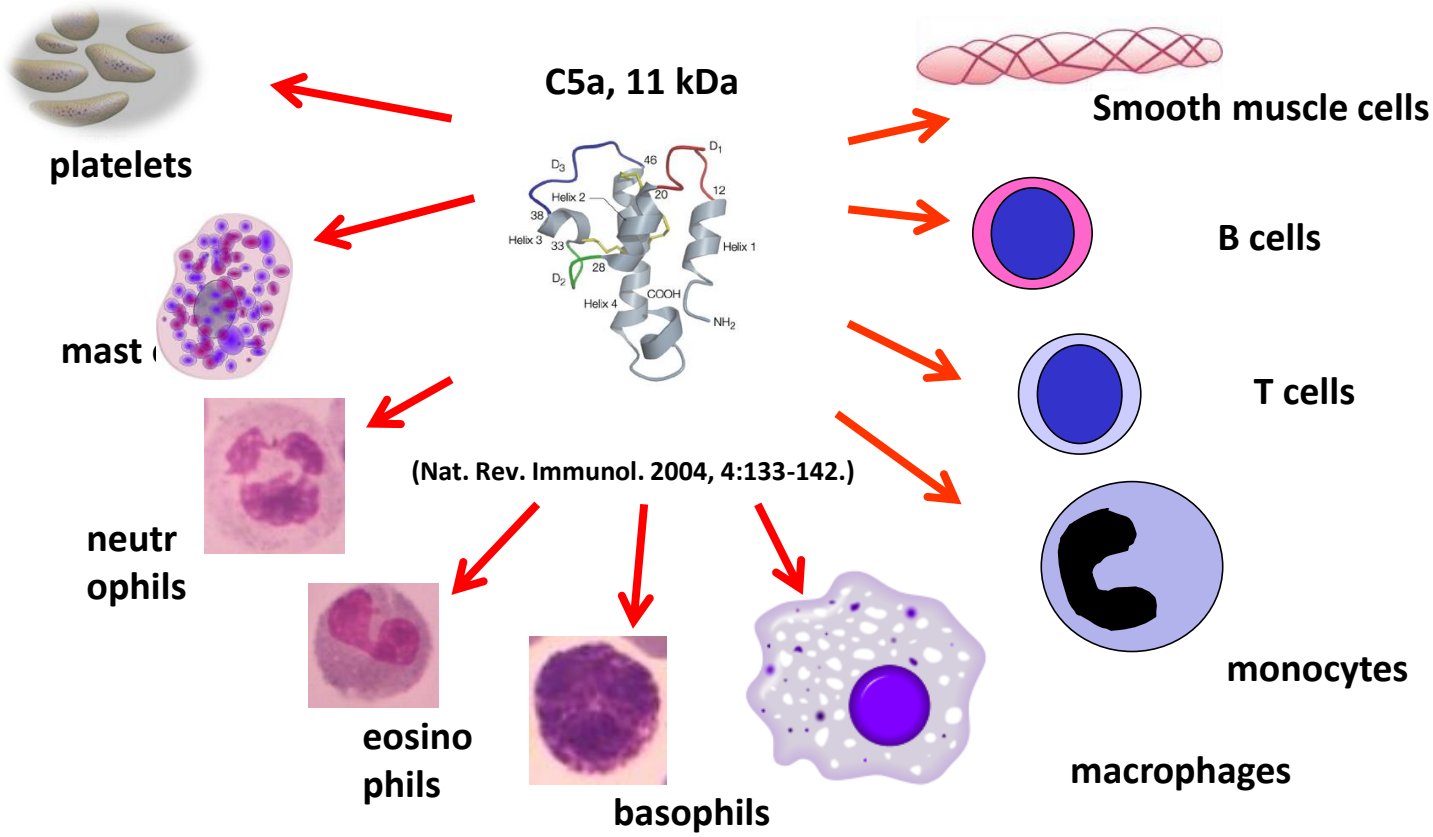
- **Rare, but serious –occasionally deadly- anaphylactic reactions may surface only in phase III-IV postmarket surveillance;**
  - ***can be fatal (in cardiac patients)***
  - ***cannot be predicted by standard allergy tests***
  - ***may lead to drug withdrawal***
- **Regulatory authorities increasingly demand experimental verification of short- and longterm immune tolerance**

C activation by liposomes
(47 y)

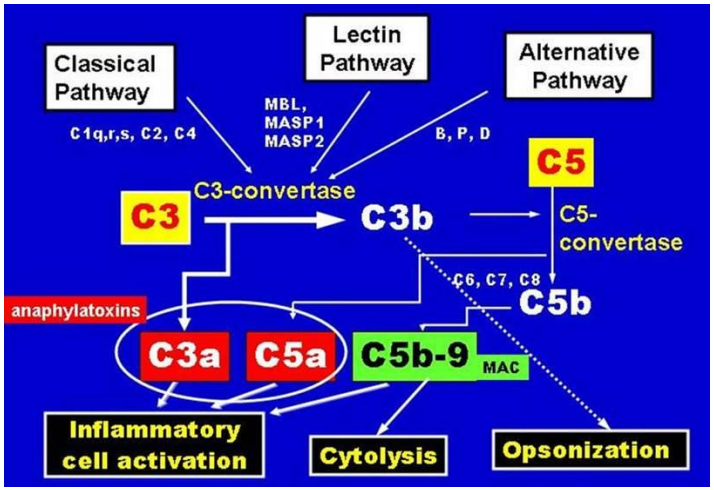
C.R. Alving, S.C. Kinsky, J.A. Haxby, C.B. Kinsky, Antibody binding and complement fixation by a liposomal model membrane, *Biochemistry 8 (1969) 1582–1587*.

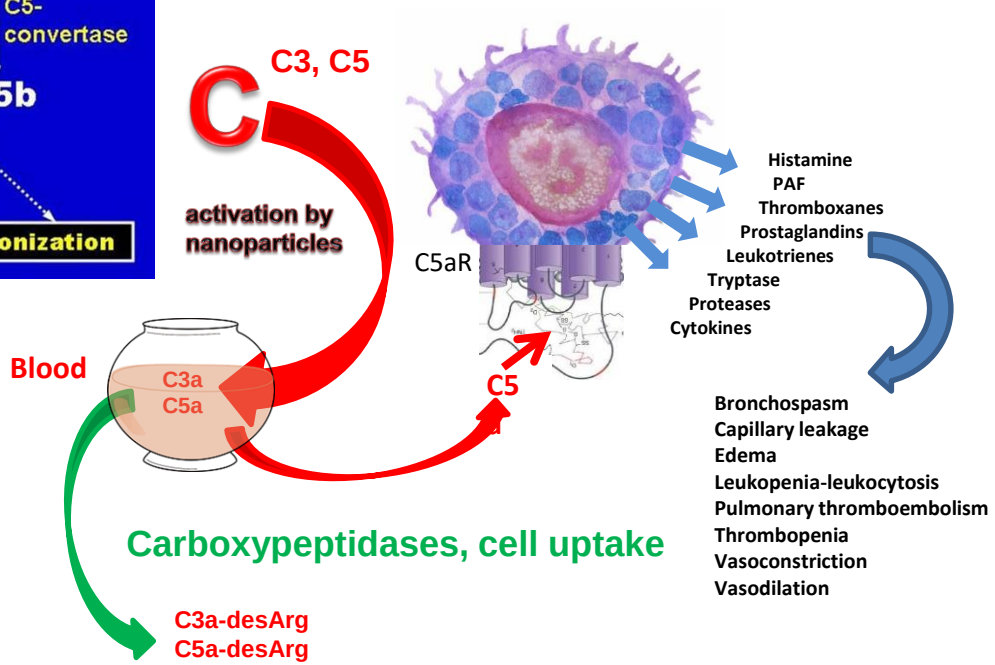


Anaphylatoxin (C3a, C5a) targets



Mechanism of CARPA



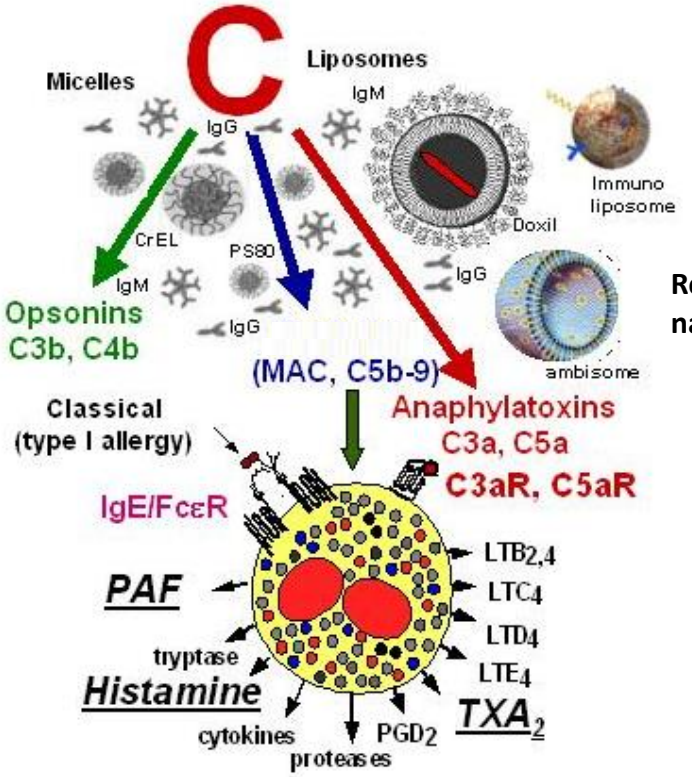


Mechanism of CARPA

C activation

Release of primary mediators

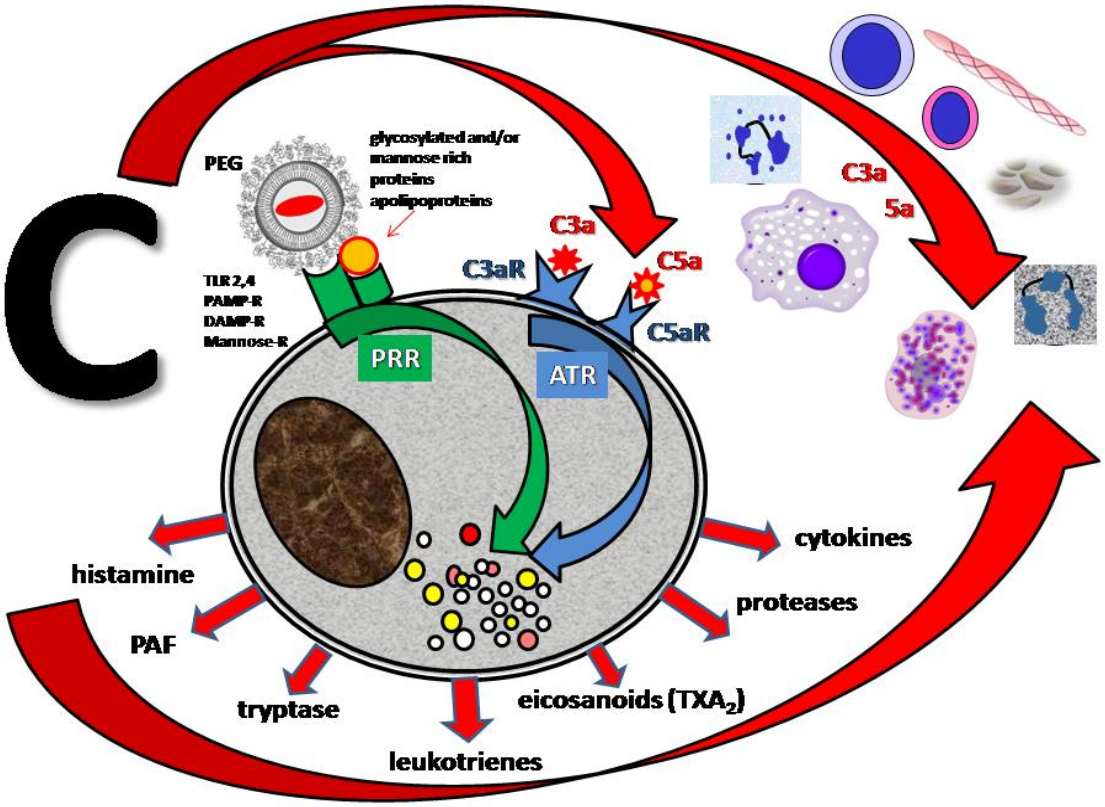
Release of Secondary mediators



Reactogenic nanoparticles

Mast cells Basophil leukocytes

The double-hit hypothesis of CARPA



Prevention and Treatment of CARPA:

Current Methods

- **Emergency measures**
  - **CPR, epinephrine, oxygen, fluids**
- **Empirical**
  - **slow infusion, break or termination of infusion**
- **Pharmacological**

Pharmacological prevention of CARPA

- **Commonly applied**
  - anti-inflammatory agents
    - Steroids
    - NSAID
      - Ibuprofen
      - acetaminophenol
  - Antihistamines
    - H1,H2

- **Potential**
  - IVIG
  - C1INH
  - Anti C5 mAb (Soliris)
  - Macrophage / RES / inhibitors
    - L-clodronate,
    - L-alendronate

Immunogenicity

•Clinical consequences of immunogenicity

- **No Consequences**
  - **Transient appearance of antibodies without any clinical significance**
- **Acute consequences**

  - Infusion reactions, anaphylactic reactions
- **Non-acute consequences**

  - Delayed-type hypersensitivity/immune complexes

  - Cross-reactivity with an endogenous counterpart

Biologicals heraeutics with immunogenicity

Product name	Protein	Indication	% Patients with immune response
ReFacto	Factor VIII	Hemophilia A	~ 30%
Intron A	Interferon α	Hepatitis C	7%
Roferon A			25%
Pegasys			9%
Pegintron			1%
Betaseron	Interferon β	Multiple Sclerosis	10-45%
Avonex			
Rebif			
Eprex	Erythropoietin	Anemia	Non immunogenic Some cases of pure red cell aplasia with Eprex
Aranesp			
Epogen			
Procrit			
Leukine	Granulocyte macrophage colony stimulating factor	Oncology	2.3% (neutralizing antibodies)
Neupogen	Granulocyte colony stimulating factor	Oncology	Non immunogenic
Neulasta			
Enbrel	TNF receptor II human Ig Fc fusion	Rheumatoid arthritis	16%
Proleukin	Interleukin-2	Oncology	74%

Immunogenicity:
unwanted humoral and cellular immune response to drugs

- **Biological/biotechnology-derived proteins, polypeptides, their derivatives, and products of which they are components, e.g., conjugates are foreign to the immune system**
- **Nanocarriers with proteins or repetitive surface conjugates can also be immunogenic**

Factors Influencing Immunogenicity

- **Patient**
  - age
  - genetic background
  - underlying disease
    - autoimmune
    - infections
    - malnutrition
    - advanced metastatic disease
    - organ failure
    - treatments
    - previous exposure to similar proteins -> pre-sensitisation
    - immune status & immunomodulating therapy

Factors Influencing Immunogenicity (2)

- **Product**
  - source of protein
  - manufacturing process (impurity profile, contaminants)
  - formulation and stability characteristics
  - degradation products, aggregates, impurities (e.g. break down products, host cell proteins
  - structural homology to other native proteins
  - post translational modifications
  - modification of the native protein (e.g. pegylation)

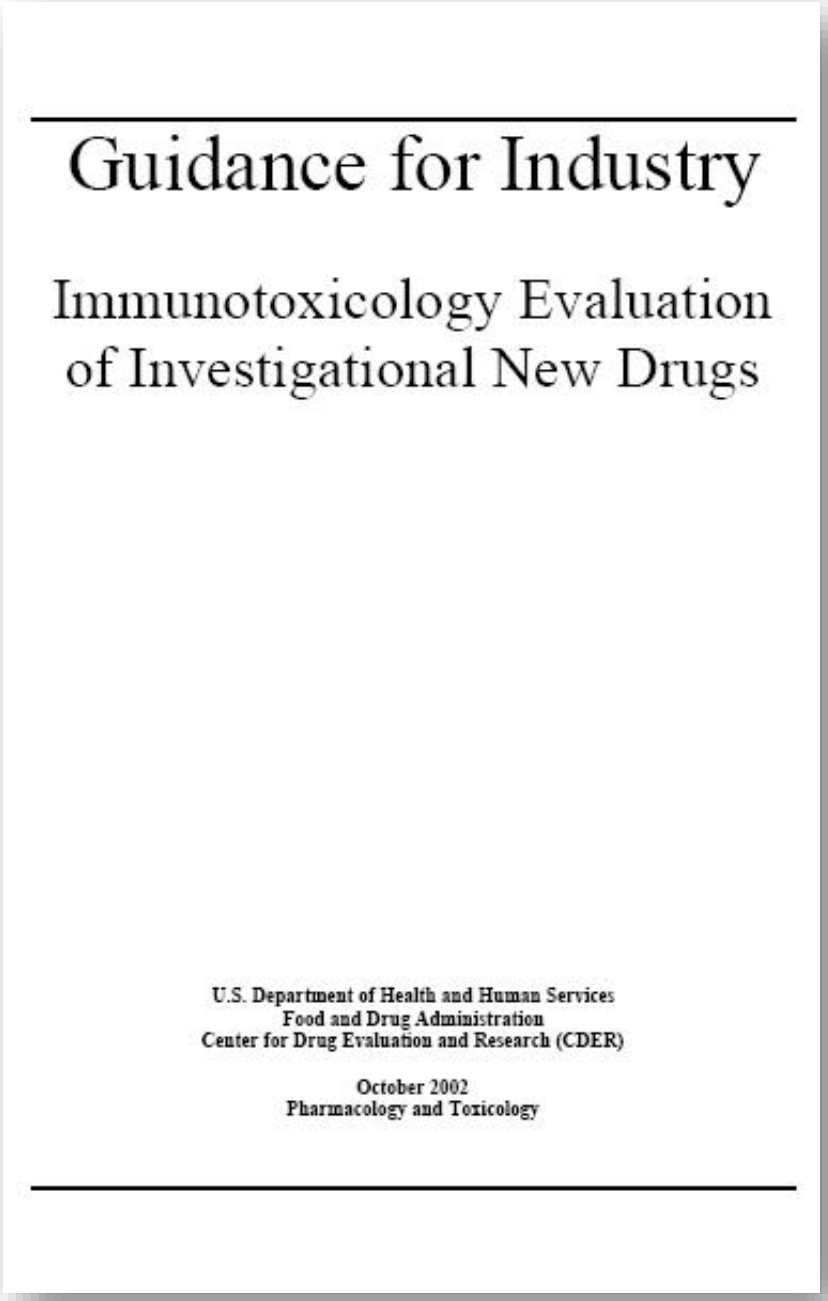
- **Treatment**
  - dose
  - route of administration
    - I.v. less immunogenic than sc. or im.
  - Schedule
    - short time treatment less immunogenic than long-term
    - continuously are less immunogenic than intermittent or re-exposure

REGULATORY ASPECTS

Tolerance of CARPA by the regulatory authorities

Depends on the risk vs. benefit ratio

- **Tolerated**
  - **terminal**
    - » **cancer**
    - » **systemic fungal infections**
- **Tolerated with restrictions**
  - **cardiovascular applications**
  - **large phospholipid doses**
  - **time limited administration**



Source: US FDA www.fda.gov

2002 - US FDA

GUIDANCE FOR INDUSTRY: IMMUNOTOXICOLOGY EVALUATION OF INVESTIGATIONAL NEW DRUGS

„Immunotoxicology is a rapidly advancing field …

New endpoints are needed for such adverse effects as systemic hypersensitivity, autoimmunity, immunogenicity and photoallergy.”

Immune Toxicity Catastrophes

1999 - Death of 18 year-old Jesse Gelsinger

at Pennsylvania University in a gene therapy trial represents a major setback to the field of gene therapy in the years to come. „No one is really sure exactly why the gene therapy treatment caused his death, but it appears that his immune system launched a raging attack on the adenovirus carrier”*

2006 - Clinical trial of a new monoclonal antibody designated TGN 1412

leaves six volunteers badly injured. Catastrophe widely publicized as „the elephant men” trial. „British regulators … concluded that TeGenero's drug TGN 1412 appeared to cause an unprecedented biological reaction in humans by stimulating the immune system”* Cytokine Storm

Drug Withdrawals

2005 - „…Palatin Technologies, the manufacturer of NeutroSpec (Technetium (99m Tc) fanolesomab) is voluntarily suspending marketing of NeutroSpec effective immediately due to serious safety concerns"

„… FDA received reports from Palatin Technologies of 2 deaths and 15 additional life-threatening adverse events in patients receiving NeutroSpec."*

*Source: US FDA
http://www.fda.gov/CDER/drug/advisory/technetium99.htm

Current in vivo immunotoxicity assays

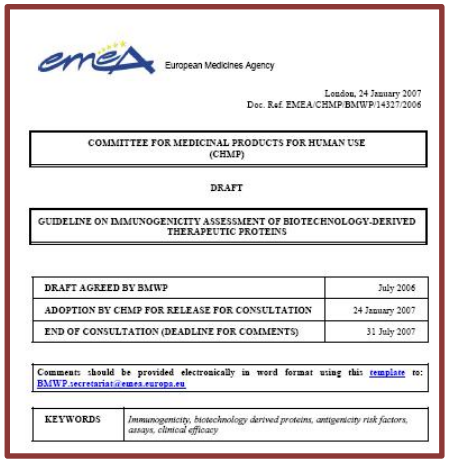
Especially warranted when standard histopathology and gross pathology identify changes in spleen, liver and other immune organs

- **The Local Lymph Node Assay (LLNA)**
  - **not recommended for nanoparticles because most nanoparticles do not cross intact skin.**
- **Lymph Node Proliferation Assay (LNPA)**
  - **modified LLNA**
  - **subcutaneous injection**
  - **only small ($\leq$ 30nm) nanoparticles have been shown to travel through the lymphatic system**
- **Antibody Forming Cell (AFC) assay**
  - **may be a good predictive tool to study nanoparticle effects on immune system function.**
- **RES uptake and macrophage function tests**

Regulatorory response to liposomal CARPA

EMA

2007



European Medicines Agency

London, 24 January 2007
Doc. Ref. EMEA/CHMP/BMWP/14327/2006

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

DRAFT

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

DRAFT AGREED BY BMWP	July 2006
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	24 January 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	31 July 2007

Comments should be provided electronically in word format using this template to: BMWP.secretariat@emea.europa.eu

KEYWORDS	*Immunogenicity, biotechnology derived proteins, antigenicity risk factors, assays, clinical efficacy*

2013

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 February 2013
EMA/CHMP/806058/2009/Rev. 02
Committee for Human Medicinal Products (CHMP)

Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

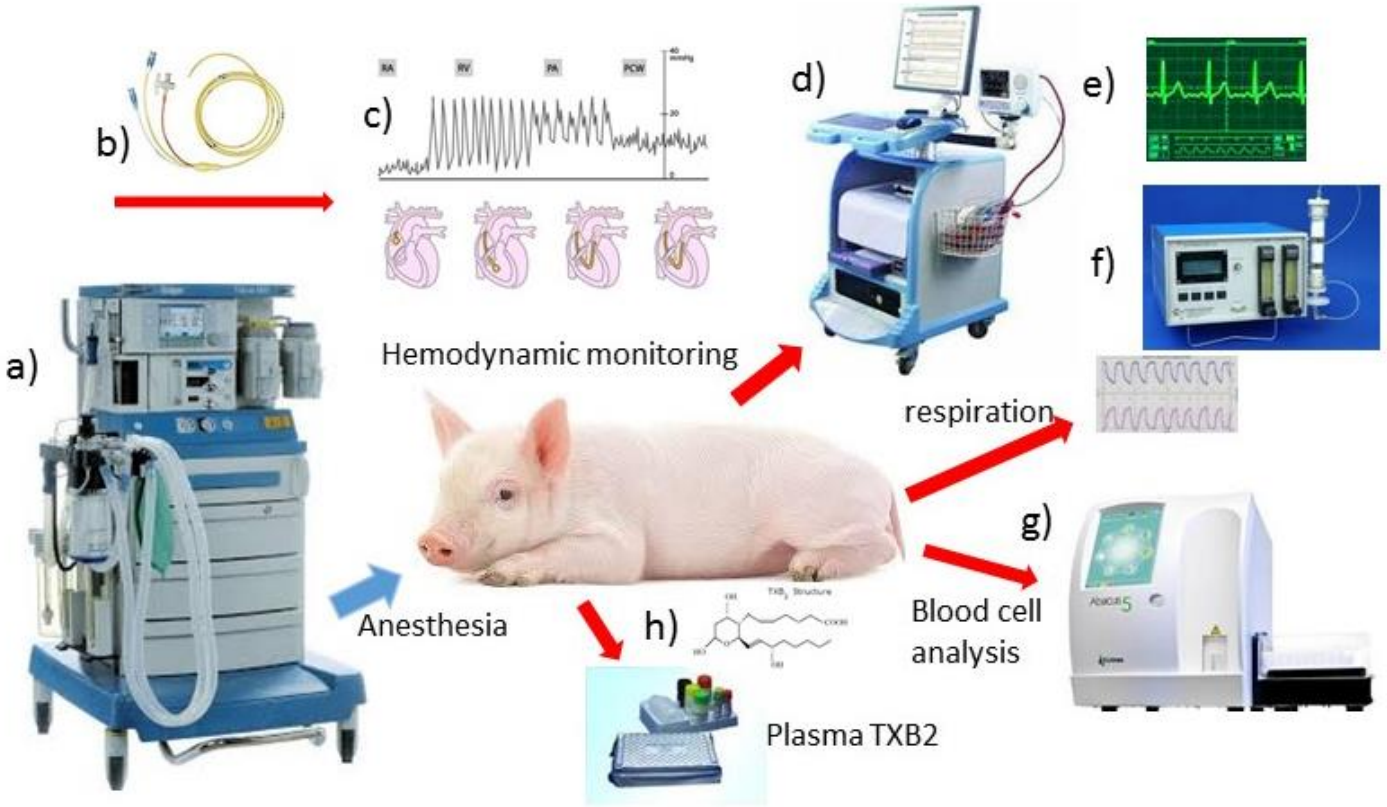
Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudoallergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event.

CARPA tests

- **In Vitro**
  - **Complement activation in human serum**
    - **C5a, C3a, SC5b-9, C4d és Bb ELISA**
  - **Basophil leukocyte activation in human blood**
    - **FACS analysis of CD203c upregulation**

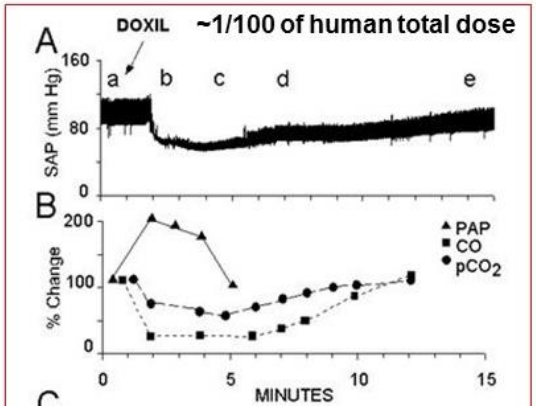
- **In Vivo**
  - Pig and dog CARPA
  - Endpoints:
    - **SAP, PAP, CO, Hr**
    - **Blood cell changes**
    - **Plazma thromboxan, histamine, leukotriene levels (ELISA)**

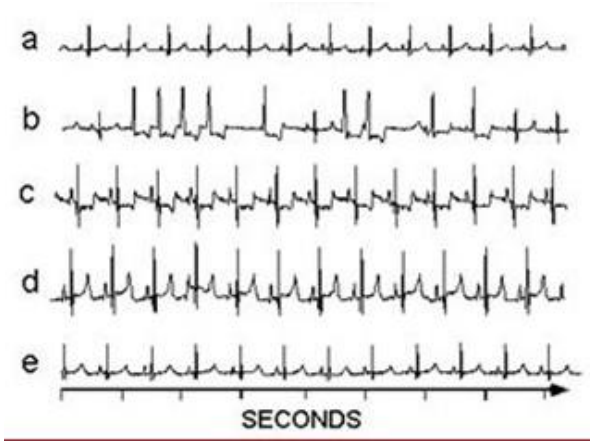
The porcine CARPA model

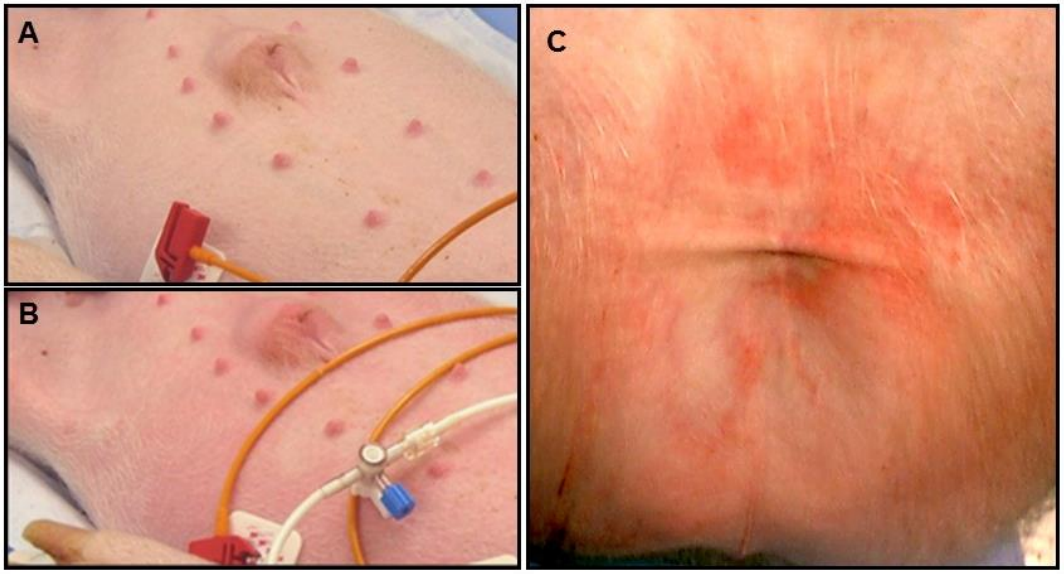


Szebeni J et al., A porcine model of complement-mediated infusion reaction to drug carrier nanosystems and other medicines ***Adv Drug Deliv Rev. 2012;64:1406-1416***

The pig model of CARPA







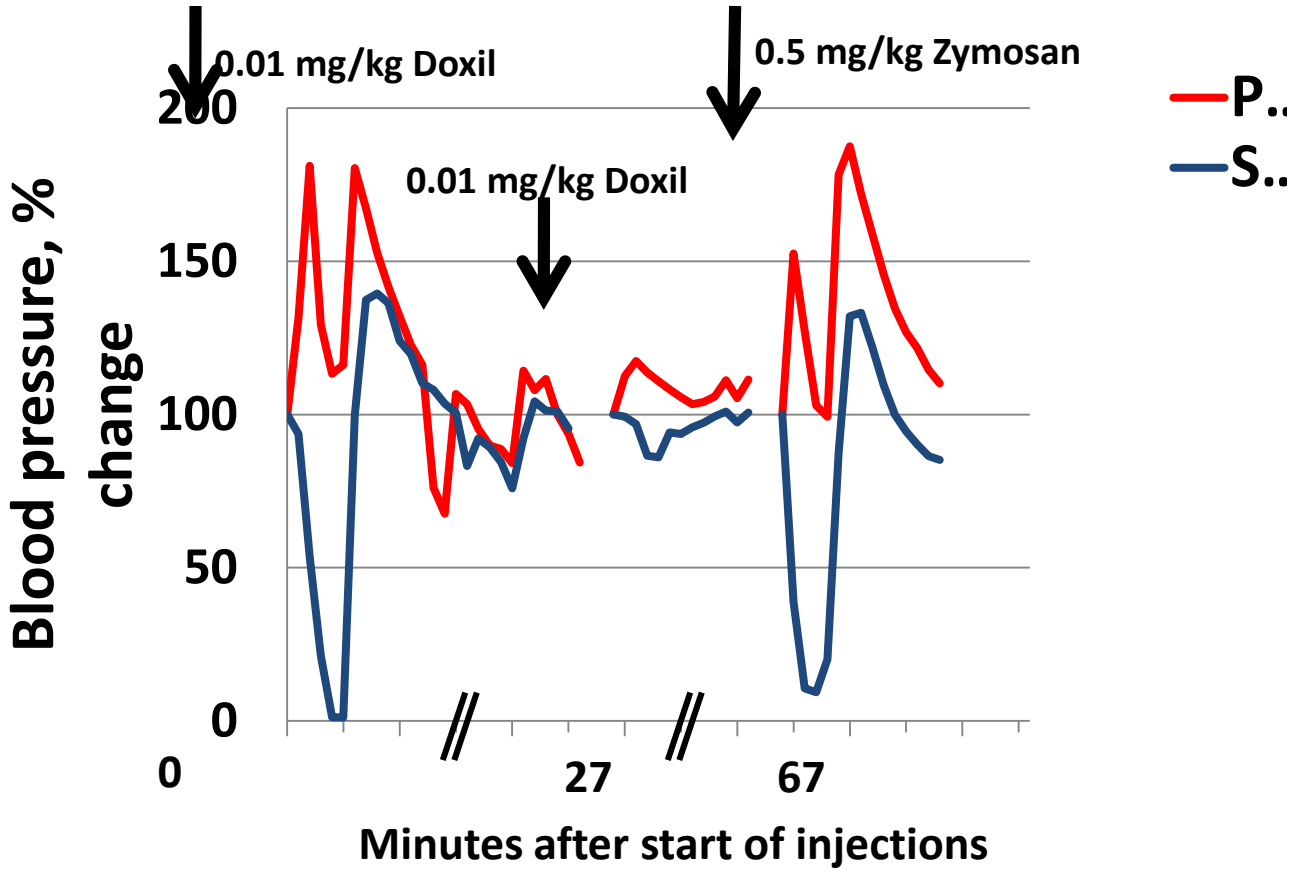
Hemodynamic alterations
- rise of PAP
- rise or decline of SAP
- declince of CO and pCO_2

Cardiac abnormalities
- tachycardia, bradycardia, arrhythmias
- ventricular fibrillation, arrest

Skin reaction
- erythema,
- rash

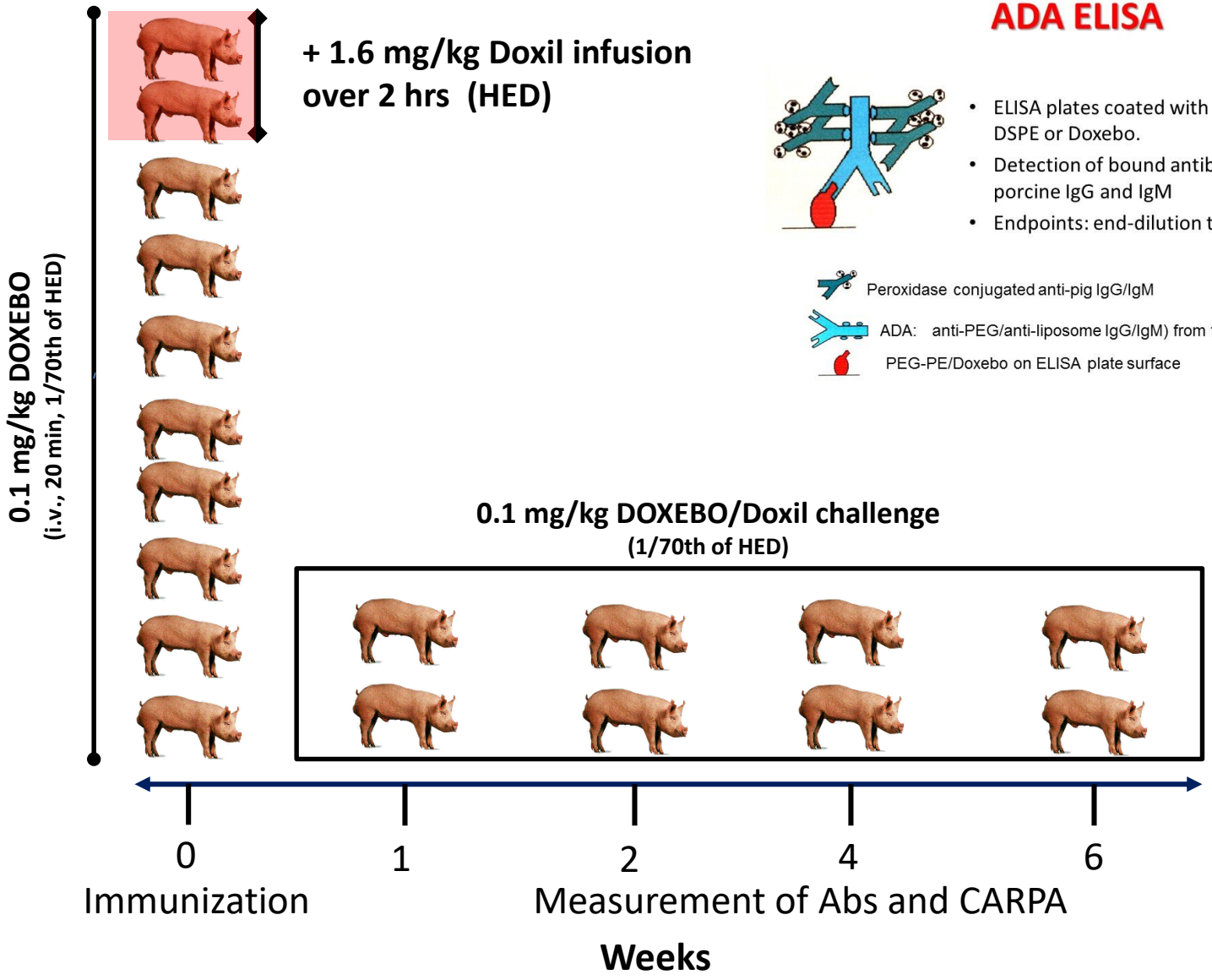
Blood abnormalities
- Leukocytosis
- leukopenia
- thrombocytosis
- thrombopenia

Doxil causes self-tolerance via tachyphylaxis



Glimpse into experiments with pigs suggesting their use in immune reactivity/immunogenicity testing

Protocol of immunization of pigs with Doxebo

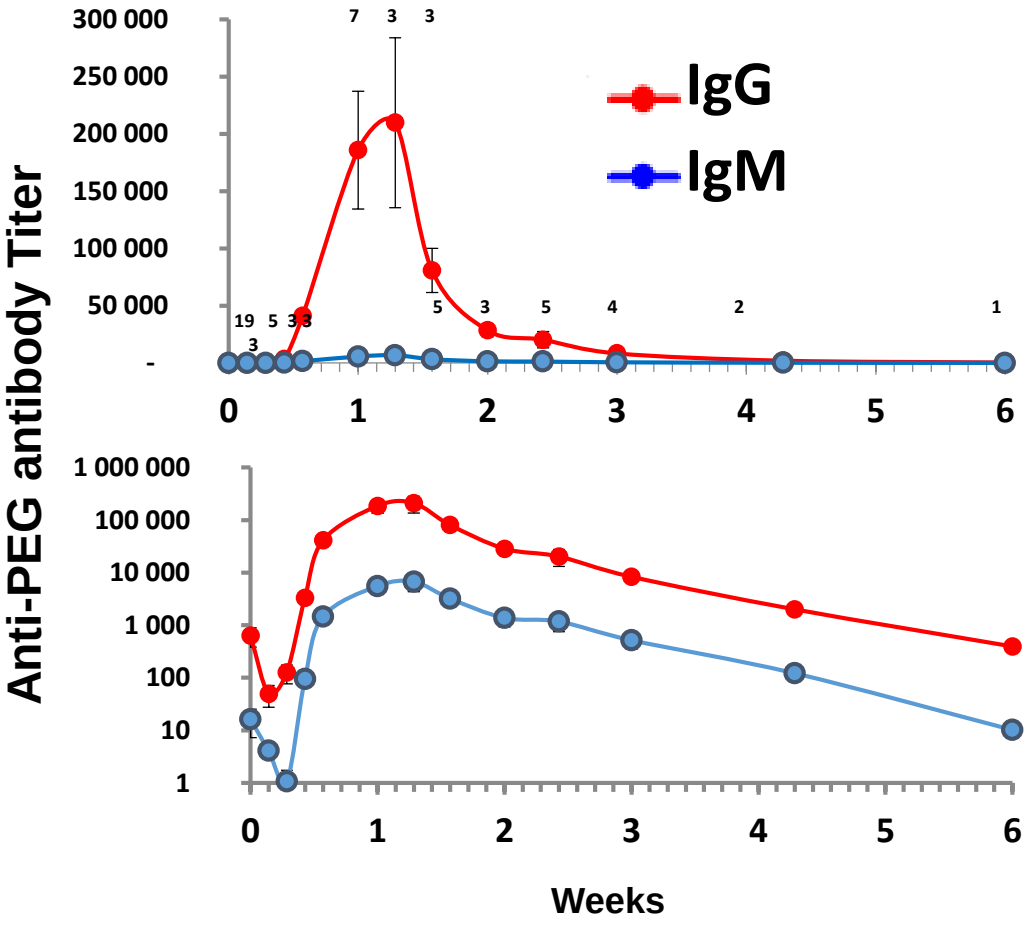


ADA ELISA

- ELISA plates coated with either mPEG2000-DSPE or Doxebo.
- Detection of bound antibodies using anti-porcine IgG and IgM
- Endpoints: end-dilution titer/arb. units

Immunogenicity of PEGylated liposomes (Doxebo) in pigs

(anti-PEG-DSPE ADA)



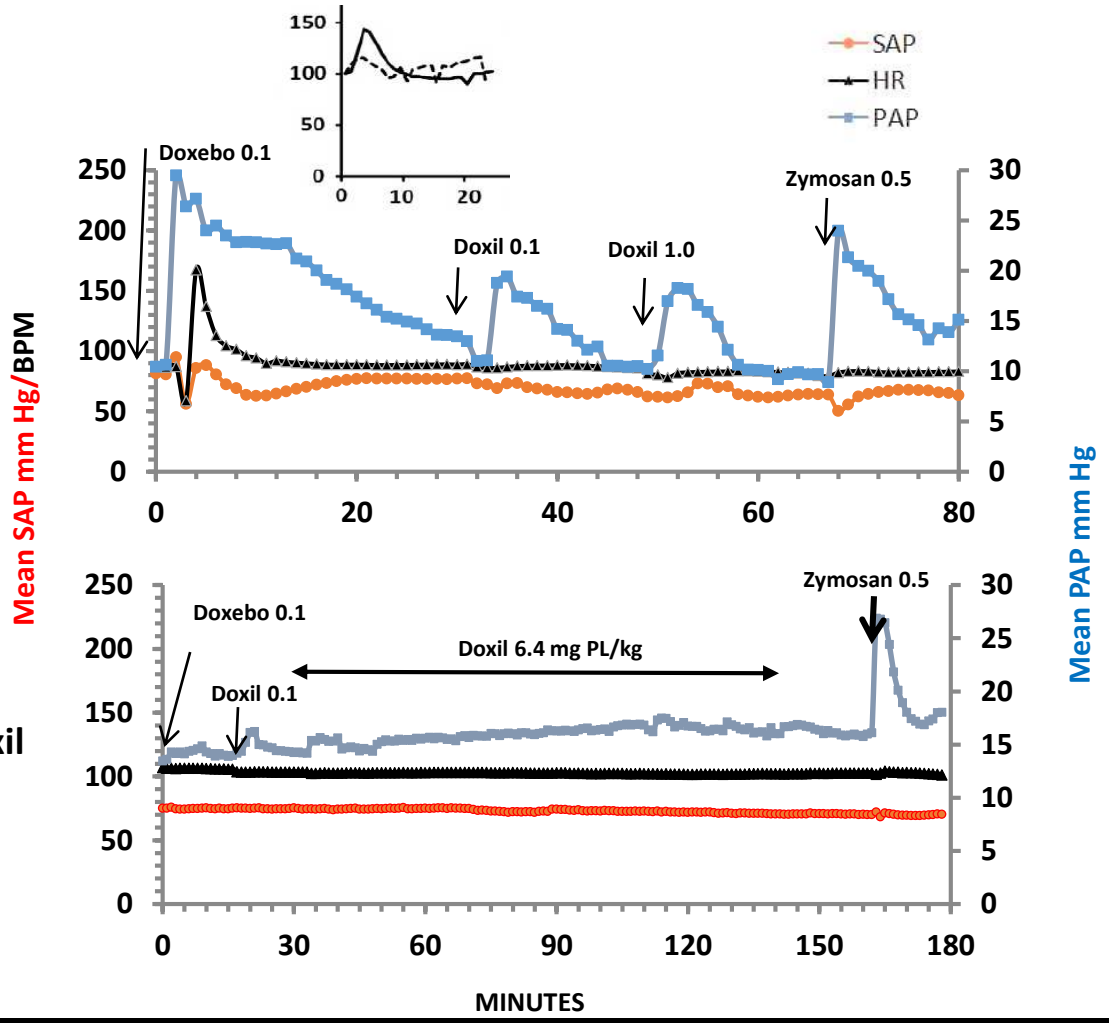
- Doxebo is highly immunogenic, leading to massive production of anti-PEG IgM and IgG antibodies
- IgM and IgG peak at day 8 ± 1
- Abs decline over 6 weeks
- IgM and IgG responses have the same kinetics
- IgM response >> IgG
- Initital titer is not zero => natural antibodies
- There is initial decrease at days 1 and 2 => Doxebo binds nAbs

Kinetics of Ab formation suggests T-cell independent B cell activation (so called type 2 immunogenicity).

Reactogenicity of Doxebo and Doxil in pigs pretreated with Doxebo with and without co-infusion of Doxil

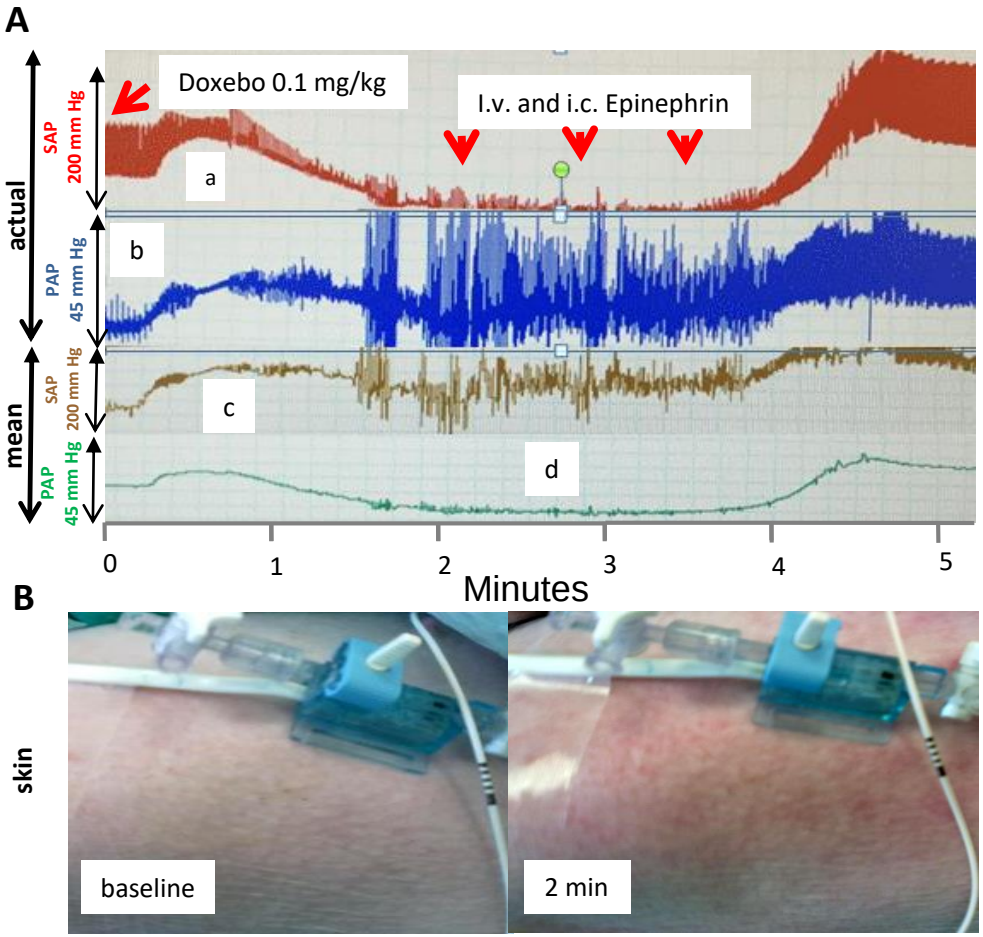
A) Doxebo only

B) Doxebo + Doxil

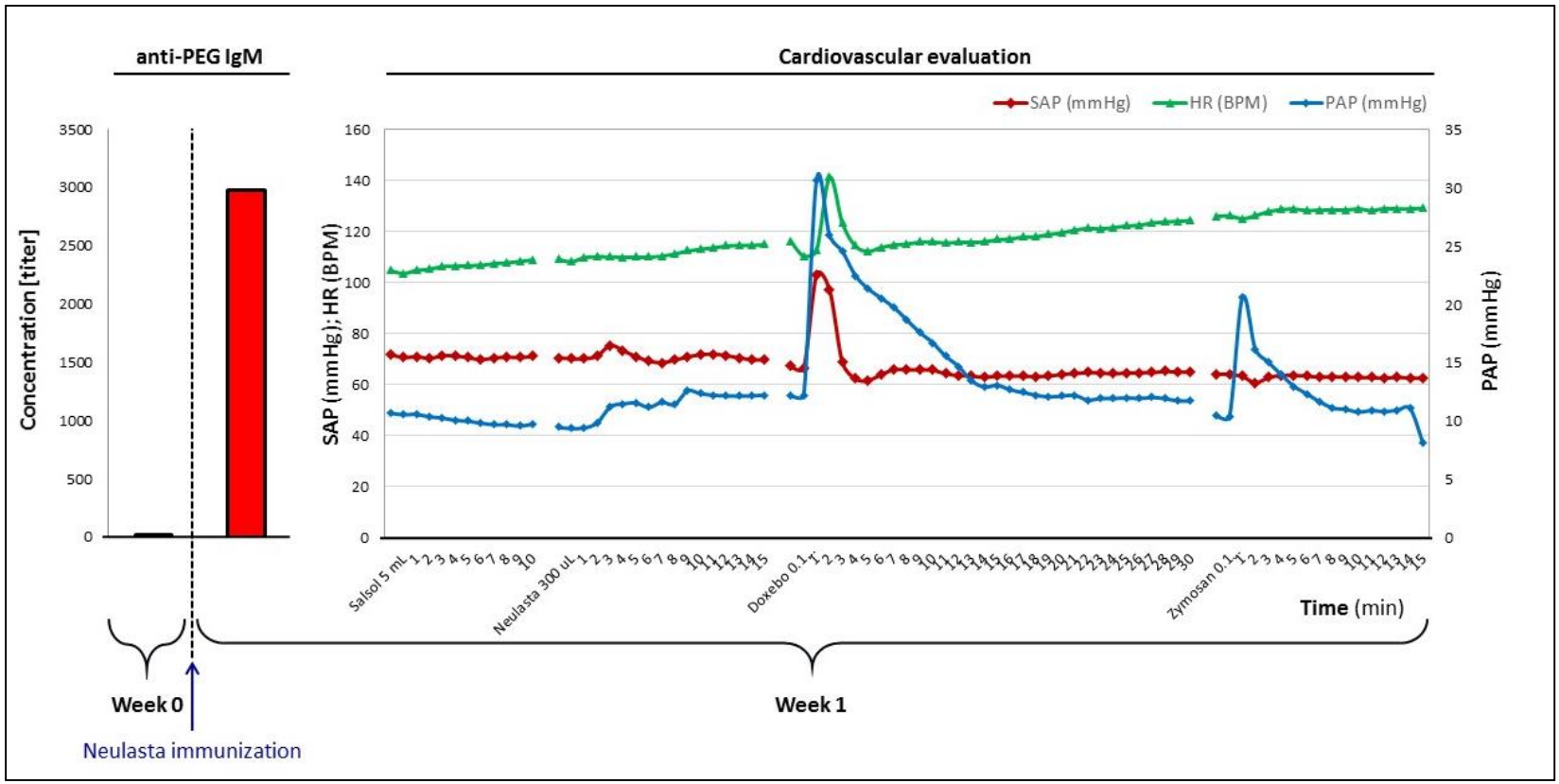


- In immunized animals the reaction to Doxebo abd Doxil is amplified
- Doxil co-treatment of animals at the time of Doxebo immunization prevents the reactogenicity of Doxebo

Doxebo can induce anaphylactic shock in immunized animals



Reactogenicity of Doxebo and Neulasta in pigs immunized with Neulasta



Immunization with neulasta produces IgM antibodies that cross-react with Doxebo, causing amplified CARPA. However, Neulasta itself does not cause reaction.
-> CARPA depends on NP size????

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Mini review

Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes

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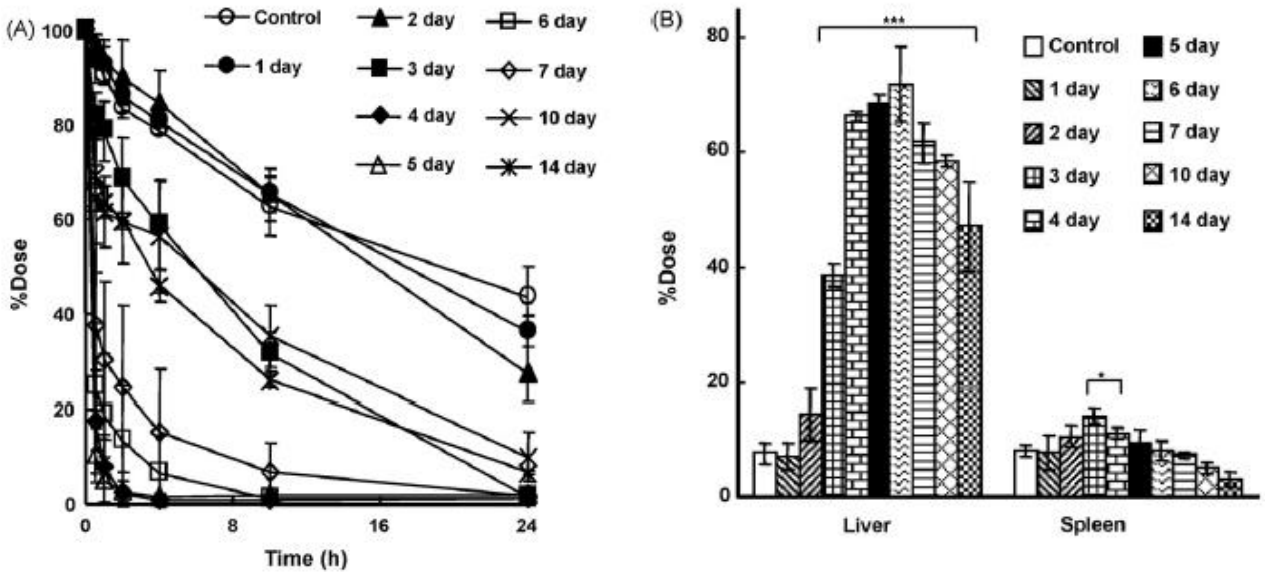
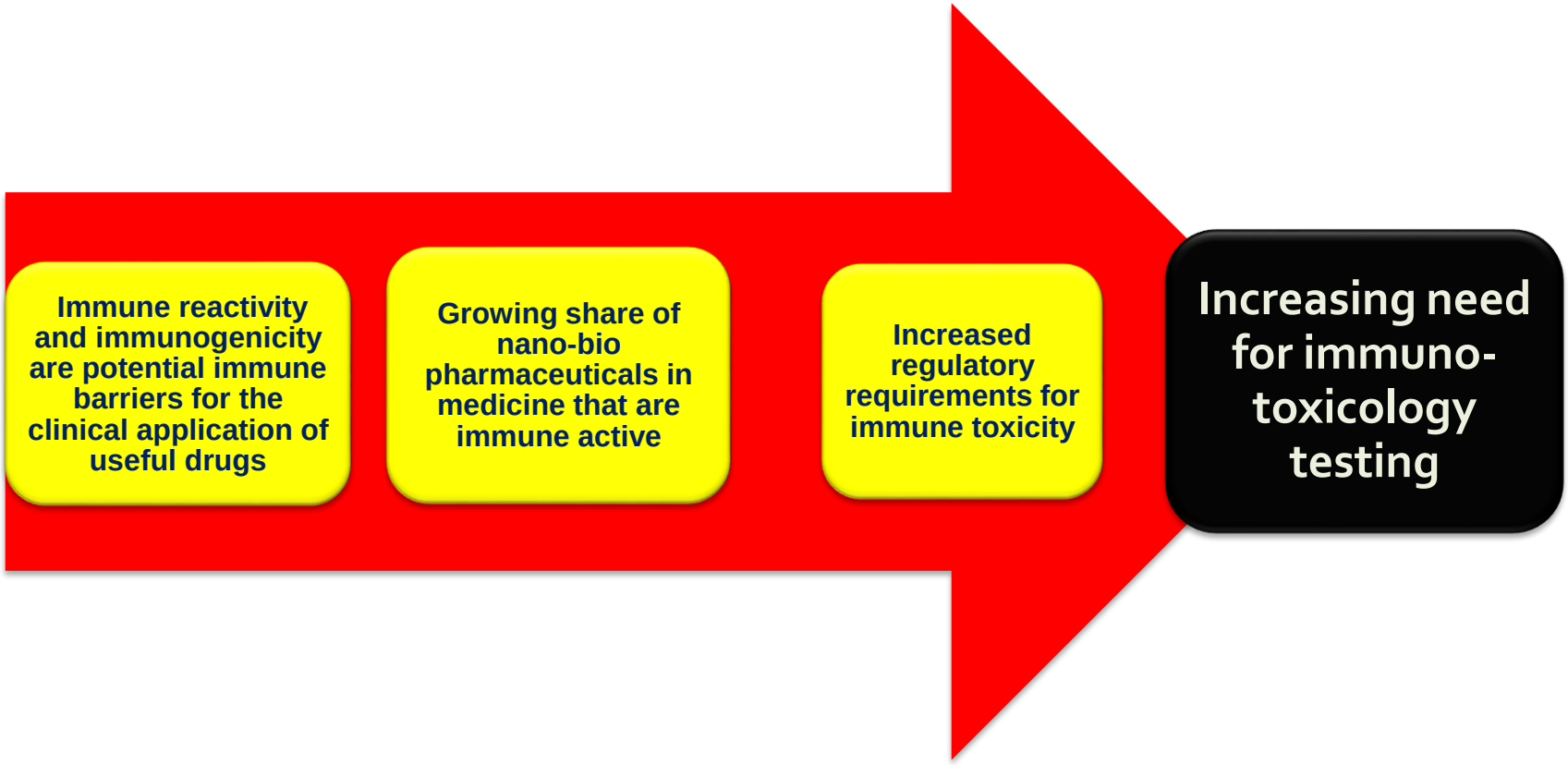


Accelerated blood clearance and enhanced organ uptake of second dose of PEGylated liposomes. Rats were pretreated with PEGylated liposomes (lipid dose, 0.001mol/kg). (A) Blood clearance of second dose of radio-labeled PEGylated liposomes (5mol/kg). (B) Hepatic and splenic accumulation at 24 h following the injection

Summary

- ***CARPA, a new immune toxicity phenomenon***
  - Brought to light by nanomedicine (liposome) research
  - Gains increasing significance in the upcoming age of nanopharmaceuticals
- ***While tolerable in cases of incurable diseases (cancer, systemic fungal infections), it may become a major safety issue in other applications***
  - Diagnostic use of nanomedicines
  - Applicaton of nanoparticles in cardiac patiens
- Tests for its prediction will likely become standard
  - Porcine model under „validation”


Immune reactivity and immunogenicity are potential immune barriers for the clinical application of useful drugs
Growing share of nano-bio pharmaceuticals in medicine that are immune active
Increased regulatory requirements for immune toxicity
Increasing need for immuno-toxicology testing

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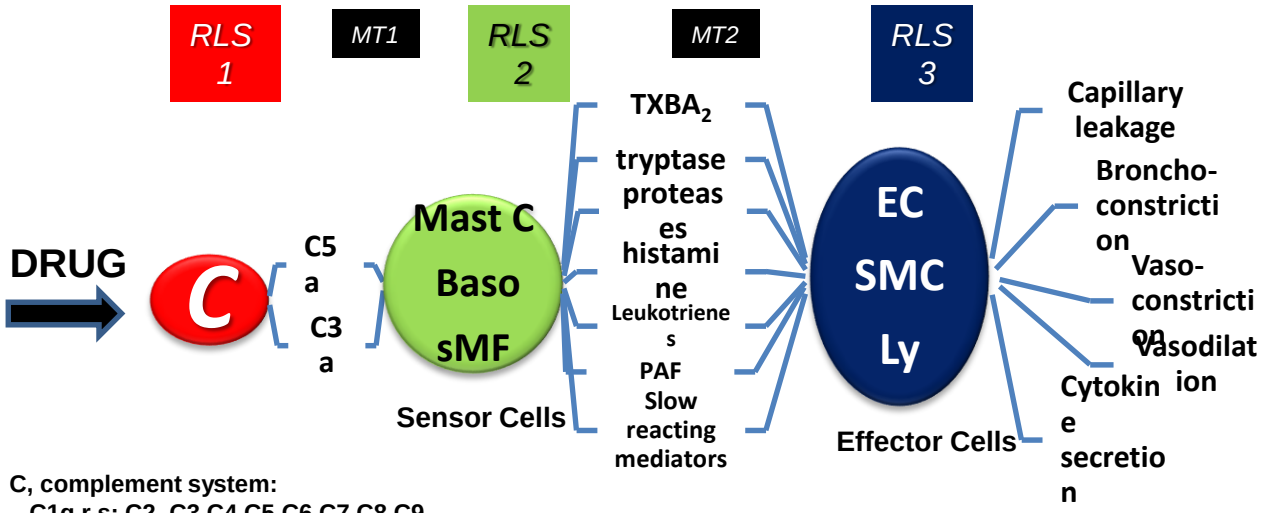
The CARPA Chain



C, complement system:
C1q,r,s; C2, C3,C4,C5,C6,C7,C8,C9
Mast C. mast cells
Baso: basophil leukocytes
sMF, secretory macrophages
EC, endothelial cells
SMC, smooth muscle cells
Ly, leukocytes

RLS: rate-limiting signaling steps: 3
Cell types involved: > 5
Mediator types (MT): 3
mediators: >> 20

Complement and CARPA assays

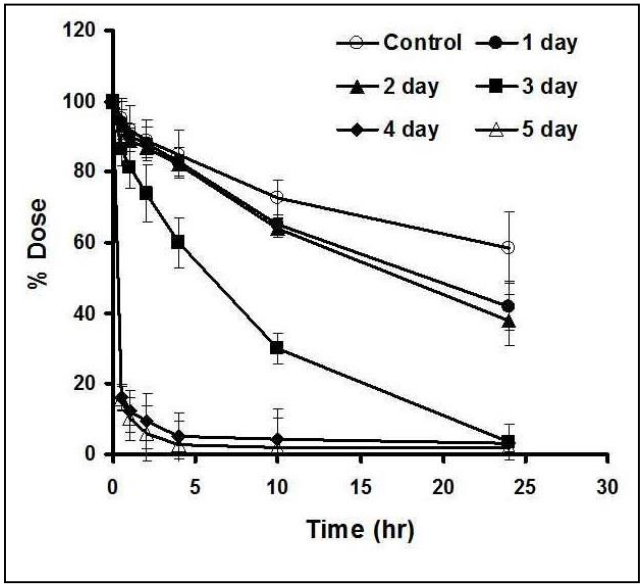
In vitro C assays in human and animal serum

- ELISA of human C3a, C5a, iC3b, SC5b-9, C4d, Bb
- All species
  - CH50
  - Pan-specific C3 from Quidel

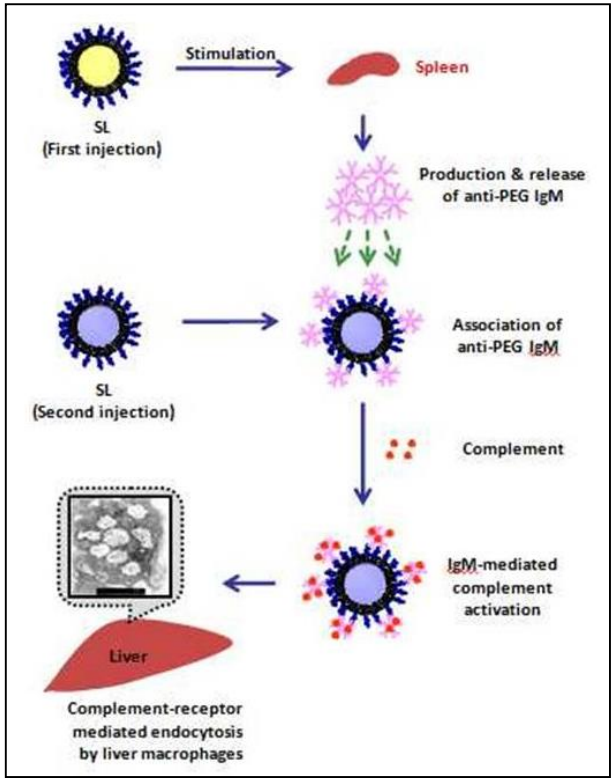
In vivo CARPA assays in pigs, dogs, rats

- Recording of hemodynamic changes after i.v. infusion of test drugs either in bolus or in infusion
- Measuring blood cell changes and plasma mediators
- Observing skin changes

The accelerated blood clearance (ABC) of PEGylated nanocarriers is due to immunogenicity



Accelerated blood clearance of a second dose of PEGylated liposomes. Rats were pretreated with PEGylated liposomes (0.001 μmol phospholipids/kg). Blood clearance of a second dose of radio-labeled PEGylated liposomes (5 μmol phospholipids/kg).



- Dams, .. Laverman, .. Storm, et al., J Pharmacol Exp Ther, 292 (2000) 1071-1079; J Pharmacol Exp Ther, 298 (2001) 607-612.
- Ishida, Shimizu,.. Koide, et al., J Control Release, 88 (2003) 35-42, J Control Release, 122 (2007) 349-355; Biol Pharm Bull, 36 (2013) 889-891; Immunobiology, 218 (2013) 725-732

Similarity and differences between stress reactions and CARPA

	Similarities	Differences
Stress	• Homeostatic function • Rapid response • Nonspecific response to variable stimuli • Leads to adaptation • Mediators are secretory products • Can lead to death • Function is to fight harmful impacts • Error-prone, causing adverse effects • Stages: Alarm->resistance-> exhaustion	• **Function**: fighting physical and psychical harm • **Noxious agents**: physical harm, emotional stress • **Time course**: from hyperacute to chronic • **Mediators**: neurotransmitters, hormones (ACTH, cortisol) • **Adverse Impact**: adrenal swelling, thymus atrophy, ulcers • **Organs involved**: neuroendocrin, cardiovascular, muscle • **Axis**: hypothalamic-pituitary-adrenal (HPA axis)
CARPA		• **Function**: fighting infections, blood clearance • **Noxious agents**: viruses, nanomedicines, antibody therapeutics • **Time course**: from hyperacute to subacute • **Mediators**: anaphylatoxins, allergomedins, cytokines • **Adverse impact**: HSRs, anaphylaxis, CARPA tetrad • **Organs involved**: cardiovascular system, skin, blood cells • **Axis**: C system-mast cells-cardiovascular system (CMC axis)

CARPA may represent a stress reaction in blood to IV-administered immune reactogenic medicines

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Review

Complement activation-related pseudoallergy: A stress reaction in blood triggered by nanomedicines and biologicals☆

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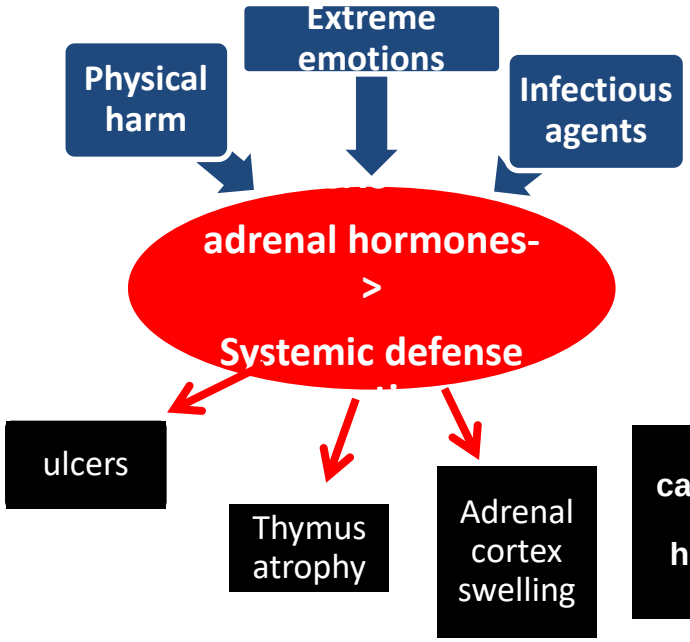
ABSTRACT

Intravenous injection of a variety of nanotechnology enhanced (liposomal, micellar, polymer-conjugated) and protein-based (antibodies, enzymes) drugs can lead to hypersensitivity reactions (HSRs), also known as infusion, or anaphylactoid reactions. The molecular mechanism of mild to severe allergy symptoms may differ from case to case and is mostly not known, however, in many cases a major cause, or contributing factor is activation of the complement (C) system. The clinical relevance of C activation-related HSRs, a non-IgE-mediated pseudoallergy (CARPA), lies in its unpredictability and occasional lethal outcome. Accordingly, there is an unmet medical need to develop laboratory assays and animal models that quantitate CARPA. This review provides basic information on CARPA; a short history, issues of nomenclature, incidence, classification of reactogenic drugs and symptoms, and the mechanisms of C activation via different pathways. It is pointed out that anaphylatoxin-induced mast cell release may not entirely explain the severe reactions; a "second hit" on allergy mediating cells may also contribute. In addressing the increasing requirements for CARPA testing, the review evaluates the available assays and animal models, and proposes a possible algorithm for the screening of reactogenic drugs and hypersensitive patients. Finally, an analogy is proposed between CARPA and the classic stress reaction, suggesting that CARPA represents a "blood stress" reaction, a systemic fight of the body against harmful biological and chemical agents via the anaphylatoxin/mast-cell/circulatory system axis, in analogy to the body's fight of physical and emotional stress via the hypothalamo/pituitary/adrenal axis. In both cases the response to a broad variety of noxious effects are funneled into a uniform pattern of physiological changes.

Physiological responses to harm

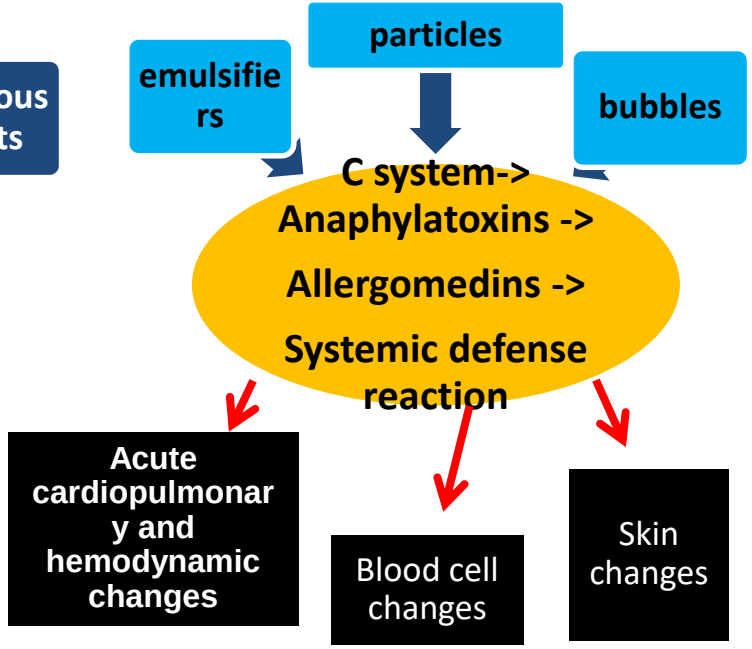
Noxious agents

Conventional stress



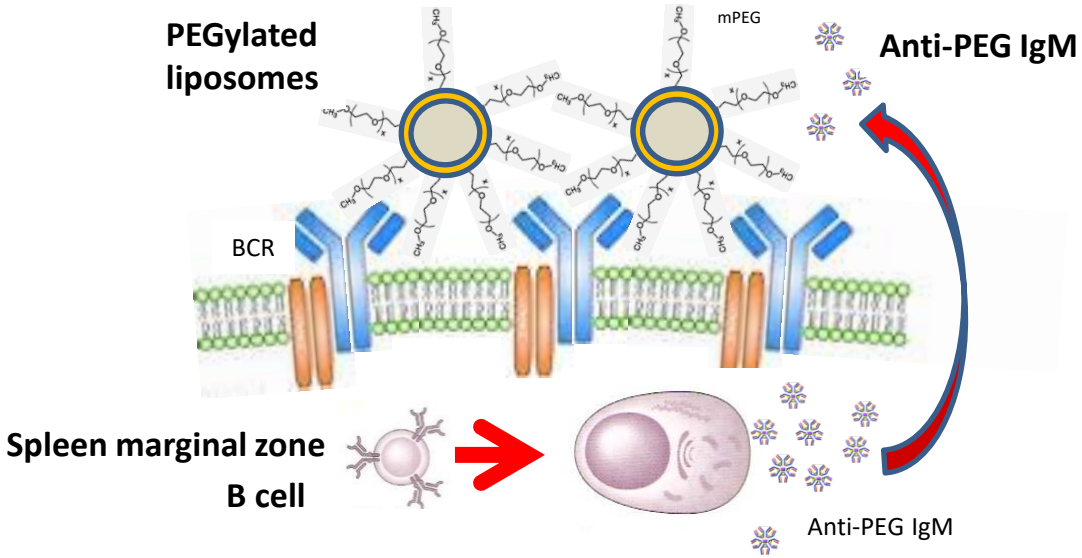
hypothalamo-pituitary-adrenal axis

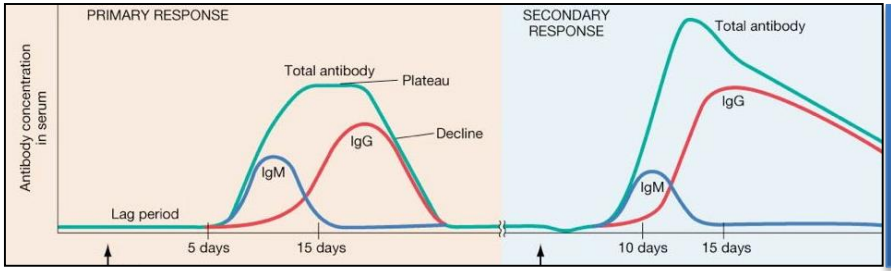
Blood stress:CARPA



C system-allergic cell-cardiovascular system axis

Mechanism of immunogenicity of pegylated liposomes





Hypothesis: Doxebo-induced immunogenicity represents a T cell independent type II (TI-2) reaction

The immune stimulatory vicious cycle caused by PEGylated nanoparticles