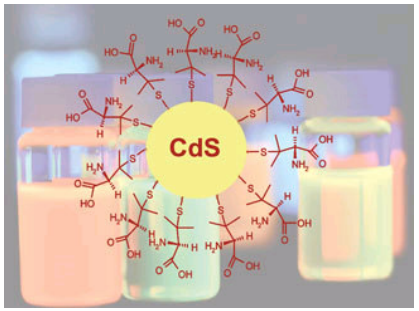

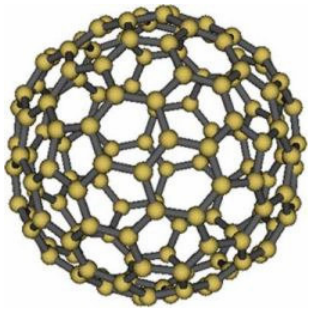
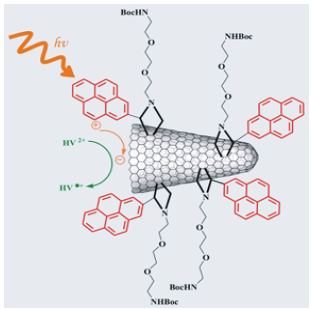

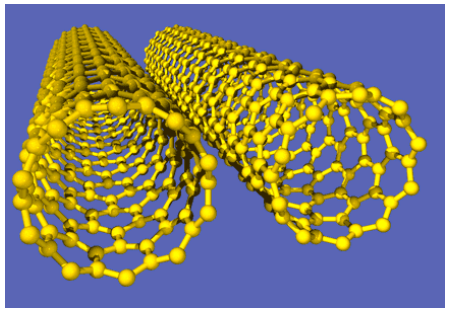

Immunology and Immunotoxicity of Nanomedicines

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Introduction

- Nanomedicines potentially useful to diagnose or treat a variety of pathological conditions: imaging nanotools, diagnostic nanochips and nanosensors, nanodelivery systems for vaccines, therapeutic proteins/peptides and antibodies, targeted nanotherapeutics…
- Widely heterogeneous group of molecular entities: dendrimers, fullerenes, quantum dots, nanotubes, nanospheres, nanohorns, nanoshells, liposomes… with highly variable physicochemical characteristics including size, shape, surface area and reactivity…
- One consistent feature: "*one or more dimensions of the order of 100 nm or less*"
- Marked toxicological as well as immunotoxicological heterogeneity to be expected across nanomedicines

Immunology of nanomedicines

- Nanoparticles, and presumably nanomedicines as well, can:
  - ✓ interact with immune cells, such as macrophages, monocytes, dendritic cells, lymphocytes
  - ✓ trigger non-specific inflammatory responses via generation of active oxygen species (oxidative burst) and release of pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-18…)
  - ✓ activate the complement cascade, and the platelets
  - ✓ facilitate antigen-specific hypersensitivity reactions via interactions with T lymphocytes and release of chemokines and immunoregulatory cytokines (IFN-γ, IL-2, IL-8, GM-CSF…)
- However, some nanoparticles can also:
  - ✓ suppress inflammatory responses
  - ✓ exert no immunomodulatory properties

- As of today:
  - ✓ no comprehensive data available on the immunological effects of nanoparticles and nanomedicines
  - ✓ no clear understanding on the exquisite mechanisms involved to account for widely differing immunological effects
- Importantly:
  - ✓ immunological effects ≠ immunotoxicity
  - ✓ immunotoxicity evaluation as part of a regulatory process
  - ✓ study of immunological effects useful primarily:
    - *to identify possible causes for concern to be addressed during dedicated immunotoxicity evaluation*
    - *to provide mechanistic clues on reported immunotoxic effects*

Immunotoxic potential of nanomedicines

- **Immunosuppression**
- **Immunostimulation/immunoactivation**
- **Hypersensitivity**
- **Auto-immunity**

Immunosuppression

- Adverse clinical consequences:
  - ✓ more frequent and most often severe bacterial, viral, fungal and/or parasitic infections due to impaired resistance to pathogens
  - ✓ more frequent virus-associated neoplasias, e.g. skin cancers and lymphomas

- Immunosuppressive nanoparticles/nanomedicines rather rarely described so far:
  - ✓ multiwalled carbon nanotubes
  - ✓ poly(D,L-lactide-co-glycolide) (PLGA) particles
  - ✓ polyhydroxy C60 (water-soluble fullerene derivative)
  - ✓ cholesterylbutyrate-conjugated lipid nanoparticles

- Nanoparticles/nanomedicines may prove to be immunosuppressive due to:
  - ✓ targeted or nanodelivery engineering to obtain intended immunosuppressive agents or formulations
  - ✓ sequestration within lymphoid organs or tissues, of variable duration, possibly resulting in immunosuppressive effects, e.g. through inhibition of phagocytosis, or immune cell functions (macrophages)
  - ✓ inadvertent immunosuppressive effects always possible…

- Preclinical evaluation:
  - ✓ regulatory setting
    - *current lack of dedicated guideline*
    - *strategy adapted from ICH S8 or ISO TS10993-20 (medical devices) guidelines ?*
  - ✓ short-term (≤ 28-day) repeat-dose toxicity study
    - *assessment based on clinical signs, standard hematology/clinical chemistry, and histology of main lymphoid organs ⇨ weight of evidence approach*
    - *at least one immune function assay (TDAR) deemed to be absolutely essential due to currently poor knowledge on immunotoxicity potential of nanomedicines*
    - *pitfalls related to consistency and quality of nanomedicines assumed to be overcome prior to conducting dedicated immunotoxicity study*

- Preclinical evaluation:

  - ✓ shortcomings and pending issues
    - *additional immune function assays (in vitro or ex vivo) deemed to be most often necessary based on known or suspected immunological effects of tested nanomedicine: e.g. macrophage function, complement activation*
    - *currently available (in vitro or ex vivo) assays poorly standardized and validated, particularly as regards safety evaluation of nanoparticles and nanomedicines*
    - *importantly, so far no in vitro assay as yet shown to be reliable predictor of immunosuppressive potential ⇨ for the time being, in vitro and ex vivo assays considered to be useful only case by case to address possible causes of concern, e.g. consequences of prolonged sequestration in macrophages or suspected complement activation*
    - *efforts to design, standardize and validate reliable (in vitro or ex vivo) assays urgently needed*

Immunostimulation/immunoactivation

- Adverse clinical consequences:
  - ✓ inflammatory complications due to cytokine release and/or complement activation ("flu-like" reactions, acute cytokine syndromes)
  - ✓ more frequent autoimmune diseases
  - ✓ more frequent hypersensitivity reactions toward unrelated allergens
  - ✓ IL-6 mediated inhibition of CYP450 pathways
- Nanoparticles may be immunostimulatory/activating
  - ✓ current development of immune nanoadjuvants for vaccines or nanoimmunotherapeutic agents
  - ✓ cytokine release induced by many nanoparticles
  - ✓ enhanced sensitization to ovalbumin through inhalation of ultrafine diesel or thiolated chitosan nanoparticles (decreased sensitization also possible ! e.g. inhalation of fullerenes)

- Preclinical evaluation:
  - ✓ regulatory setting: no dedicated guideline
  - ✓ short-term (28-day) repeat-dose toxicity study
    - *same design as assessment of immunosuppressive potential potentially helpful ?*
  - ✓ ex vivo or in vitro assays focused on possible adverse effects
    - *cytokine release assays using human cell lines or human blood*
    - *complement activation, lymphocyte proliferation…*
    - *standardization and validation to nanomedicines urgently needed*
  - ✓ safety immunopharmacology studies ?
    - *selected case by case to address causes for concern*
    - *e.g. rodent models of allergen-induced asthma or IgE-dependent anaphylaxis*

Hypersensitivity

- Adverse clinical consequences:
  - ✓ immune-mediated hypersensitivity reactions
    - anaphylaxis, immuno-allergic cytopenias, T-cell mediated organ-specific hypersensitivity reactions (pneumonitis, hepatitis, nephritis…), sometimes life-threatening
    - seemingly not reported so far
  - ✓ nonimmune-mediated (pseudo-allergic) hypersensitivity reactions
    - direct (non-specific) histamine release or complement activation (resulting in generation of the anaphylatoxins C3a and C5a, and formation of the membrane attack complex C5b-9)
    - no prior sensitizing contact required (possible first-dose reaction)
    - somewhat different clinically from IgE-dependent anaphylaxis

- Concern regarding immune-mediated hypersensitivity related to:
  - ✓ possible intrinsic immunogenicity of nanomolecules (but low molecular weight and non-peptidic structure)
  - ✓ immunogenicity of nanomolecules resulting from inadvertently adsorbed chemicals or purposely bound chemicals or drug moiety (targeted engineering)
- Concern regarding nonimmune-mediated hypersensitivity related to:
  - ✓ pseudo-allergic reactions through complement activation reported with liposomes (and Cremophor EL®)

- Preclinical evaluation:
  - ✓ regulatory setting: no dedicated guideline
  - ✓ preclinical assessment suspected to be less tricky than with therapeutic proteins
    - *lack of humanization of current nanomedicines (animal models theoretically applicable)*
    - *lack of metabolic activation (no involvement of reactive metabolites)*
    - *prediction still hampered by many limitations and pitfalls*
  - ✓ models and assays selected case by case

- Preclinical evaluation:
  - ✓ models and assays
    - *systemic or cutaneous passive anaphylaxis to detect specific IgE in previously treated rodents or guinea-pigs*
    - *basophil activation assay (flow cytometry), possible with human blood samples*
    - *histamine release assay, also possible with human blood samples*
    - *complement activation:*
      - ex vivo or in vivo measurement of anaphylatoxins (C3a, C5a), C3a-desarg, or SC5b-9 from human blood samples
      - dedicated pig and dog models of liposome-induced complement activation (rodents relatively insensitive)

Autoimmunity

- Autoimmunity can manifest as systemic (e.g. lupus) or organ-specific (e.g. myasthenia) disease
- Only elusive data available on possible link between autoimmunity and exposure to ultrafine particles
- Autoimmunity potential beyond reach of any reliable prediction to date

Conclusion

- Currently available immunological data deemed to suggest immunotoxicity potential as an (at least theoretical) cause for concern with most, if not all nanomedicines ⇨ systematic preclinical immunotoxicity evaluation to be recommended
- Most current models and assays presumably applicable to some extent even though standardization and adaptation to nanomedicines evaluation obviously needed
- Specificities and modalities of the immunotoxicity evaluation of nanomedicines to be identified and validated for regulatory purpose