GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

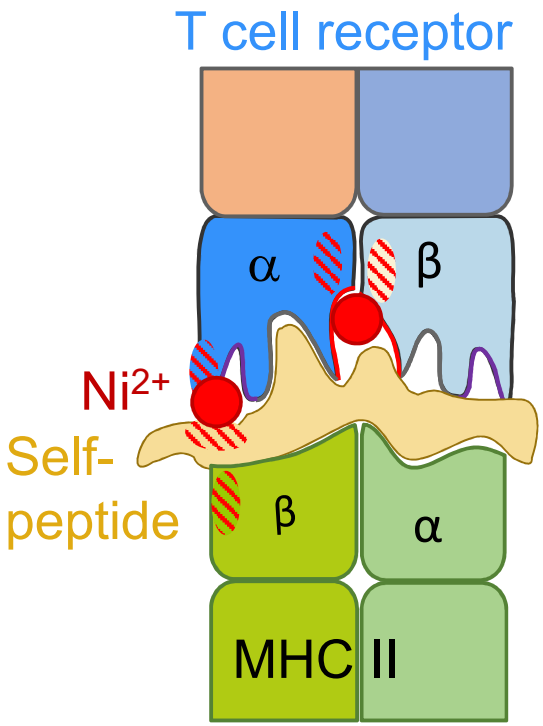
20 YEARS

BfR
German Federal Institute for Risk Assessment

Sensitizing properties of nanoparticles

Katherina Siewert
Dermatotoxicology Study Centre
Department of Chemical and Product Safety

4th Joint Symposium on Nanotechnology
30th May 2022



Adapted from Riedel 2021

Research at the Dermatotoxicology Study Centre

Dr. Katherina Siewert - Allergies

1,5% hair dye
(p-phenylendiamine, PPD)

Dr. Ines Schreiver - Tattoos

Pigment red22

35% of allergic reactions

Serup, 2019

Nickel 11%

Chromium 1,8%

Aparicio-Soto, 2020
Aparicio-Soto, 2021
Curato, 2022
Riedel, 2021
Weiß, 2021

BfR

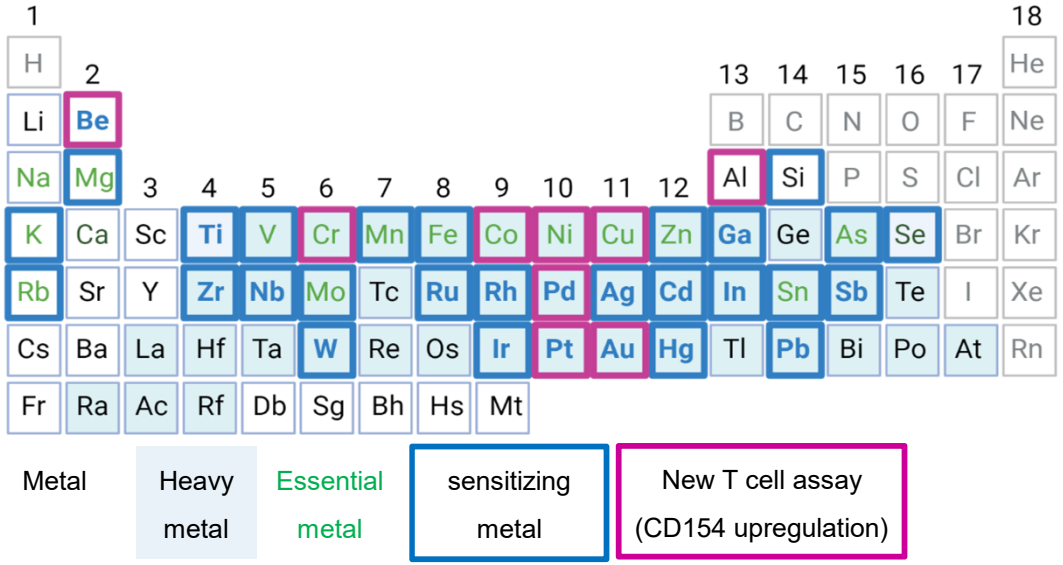
The world of sensitizing substances

~1 in 5 chemical sensitizes Basketter, 2010 cas registry: 264 million substances 27.5.2022

~20 - 27% of individuals allergic (patch testing) Alinaghi, 2019; Diepgen, 2016

Nanomaterials may contain sensitizers, adsorb allergenic proteins 1805 proteins, www.allergen.org

or modulate immune responses



Adapted from Riedel, 2021

Objective

predict & diagnose allergies

Schemas created with BioRender.com

Diagnosis & monitoring of allergy prevalence

To date, BfR is not aware of any case in which damage to health has been proven to have been caused by nanomaterials contained in a consumer product.

https://www.bfr.bund.de/de/fragen_und_antworten_zu_nanomaterialien-8552.html

- But: limitations of epicutaneous testing

lack of patch testing preparations, positive results not linked to clinical symptoms, lack of skin migration

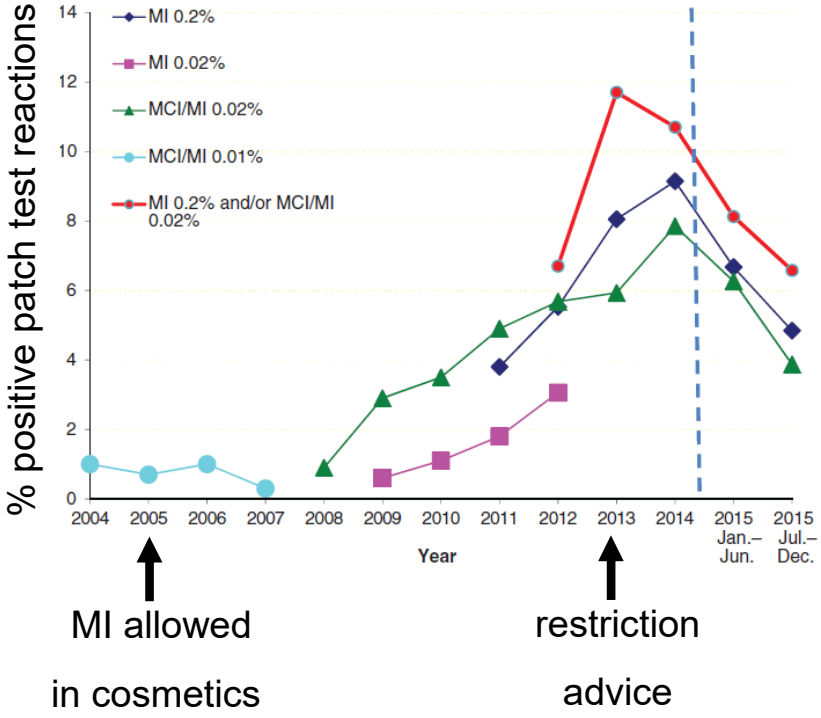
→ Challenges population allergy prevalence surveillance

(by IVDK, ESSCA)

- Alternative diagnostic *in vitro* tests still largely experimental/complementary

What we want to avoid: epidemics

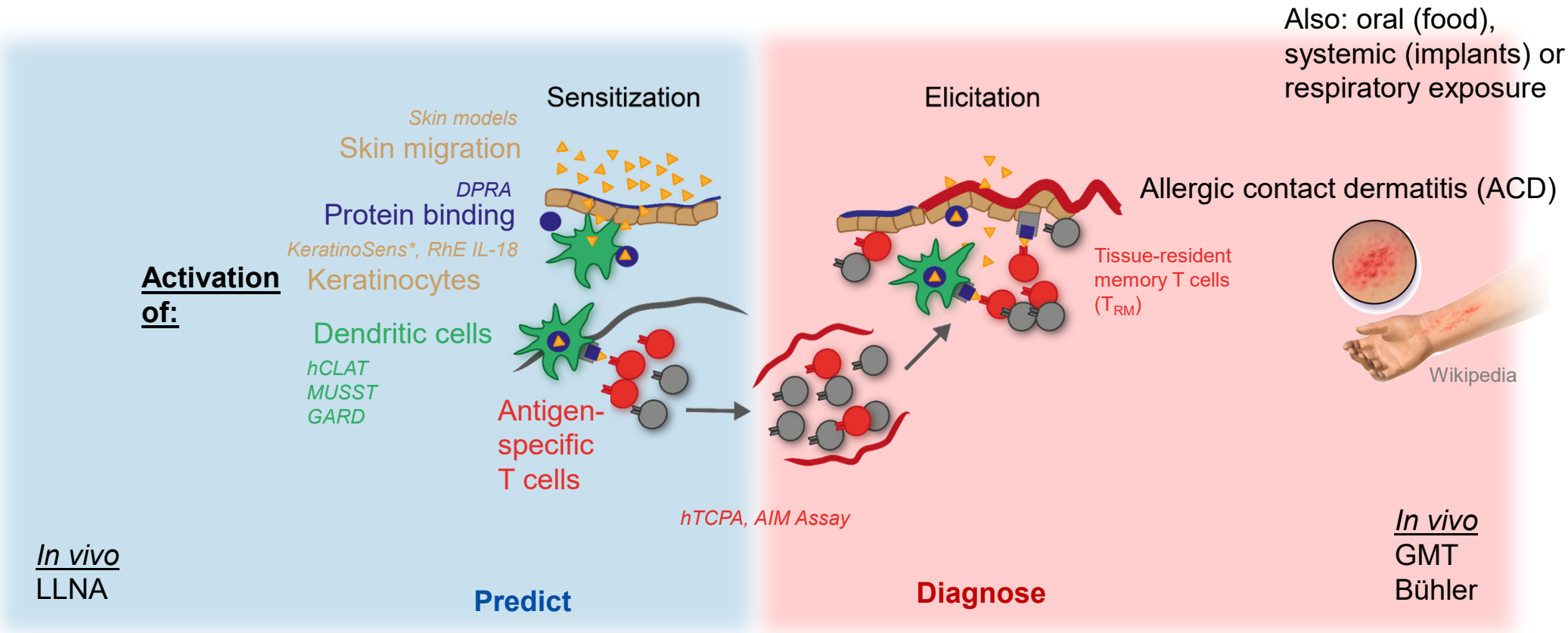
(e.g., to methylisothiazolinone (MI)



Urwin, 2017

IVDK – Informationsverbund dermatologischer Kliniken, **ESSCA** - European Surveillance System on Contact Allergies

Predictive tests for sensitizing properties address pathomechanism of (skin) sensitization



*Only assay currently adopted for nanomaterials (NanoHarmony)

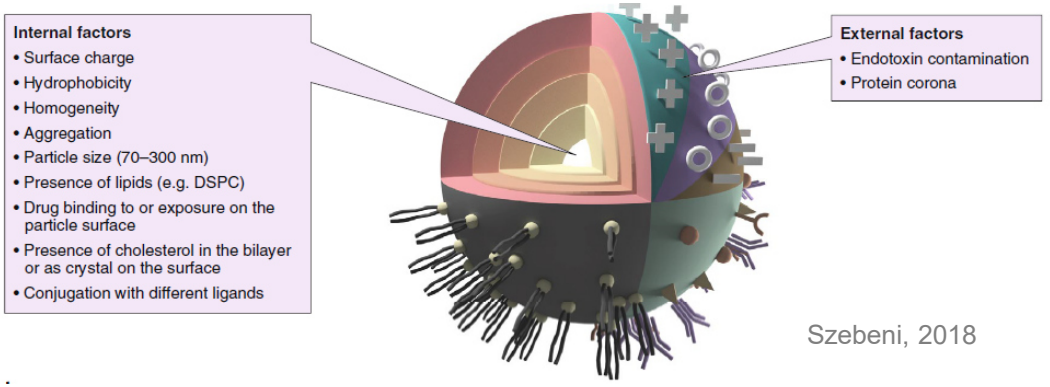
DPRA – direct peptide reactivity assay, **RhE** – recombinant human epidermis, **hCLAT** – human cell line activation test, **MUSST** - Myeloid U937 Skin Sensitization Test, **GARD** - Genomic Allergen Rapid Detection, **hTCPA** – human T cell priming assay, **AIM** – activation-induced marker, **LLNA** – local lymphnode assay, **GMT** – guinea pig maximization test

Examples for nanomaterial sensitization – antigen-specific reactions

- ceramic-, metal-, carbon and polymer-based exosomes; liposomes; scaffolds; +/-coatings or ligands, material absorbed or incorporated

SARS-CoV-2 vaccine Szebeni, 2020
>1 billion mRNA vaccinations
1 in 2.5-4.5 million: anaphylactic reactions

Potential antigens: spike protein, PEG
(or non-IgE mechanism)



Szebeni, 2018

Negative guinea pig test Ema, 2011
single-walled carbon nanotubes (SWCNTs)
multi-walled carbon nanotubes (MWCNTs)

Negative LLNA Park, 2011
SiO_2, TiO_2 Lee, 2011

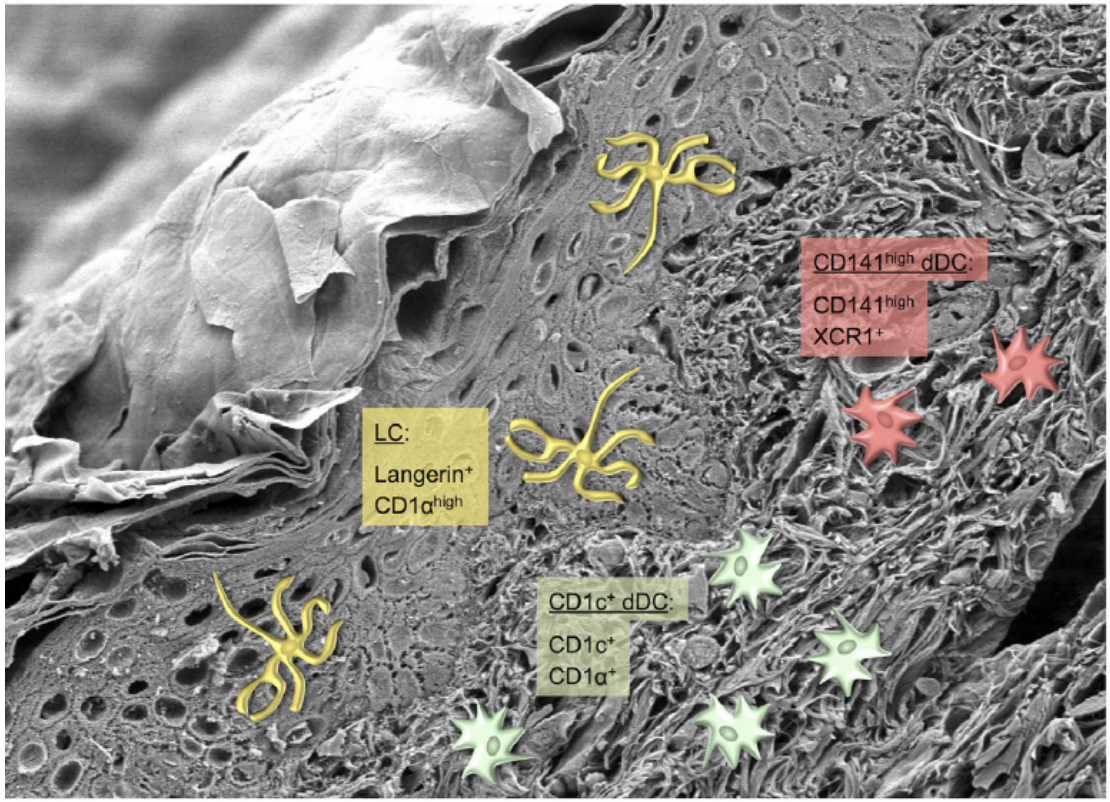
Metals in NP Swinnen, 2013
associated with airborne ACD

But: STAT6-dependent exacerbation of house dust mite-induced allergic airway disease in mice Ihrie, 2021

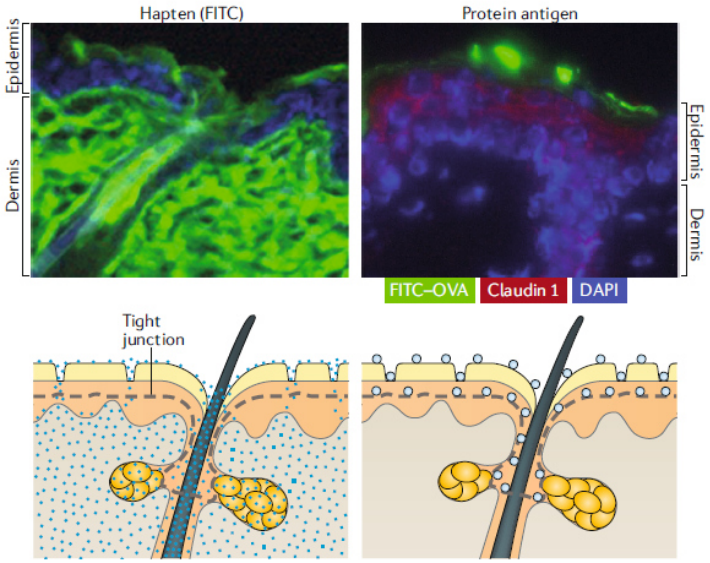
diesel exhaust particles (DEP) do the same Brandt, 2015
ZnO NP agument IgE in atopic dermatitis model Ilves, 2014
TiO_2 NP increase DNCB sensitization Smulders, 2015

Nanomaterials hardly migrate through intact skin

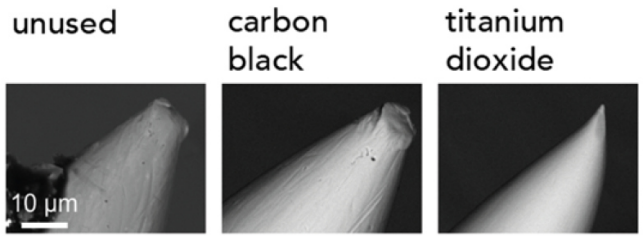
- Tattoo inks are negative in patch tests Serup, 2014
- Migration enhanced on damaged skin (flexes) Tinkle, 2004



Clausen, 2015



Kabashima, 2019



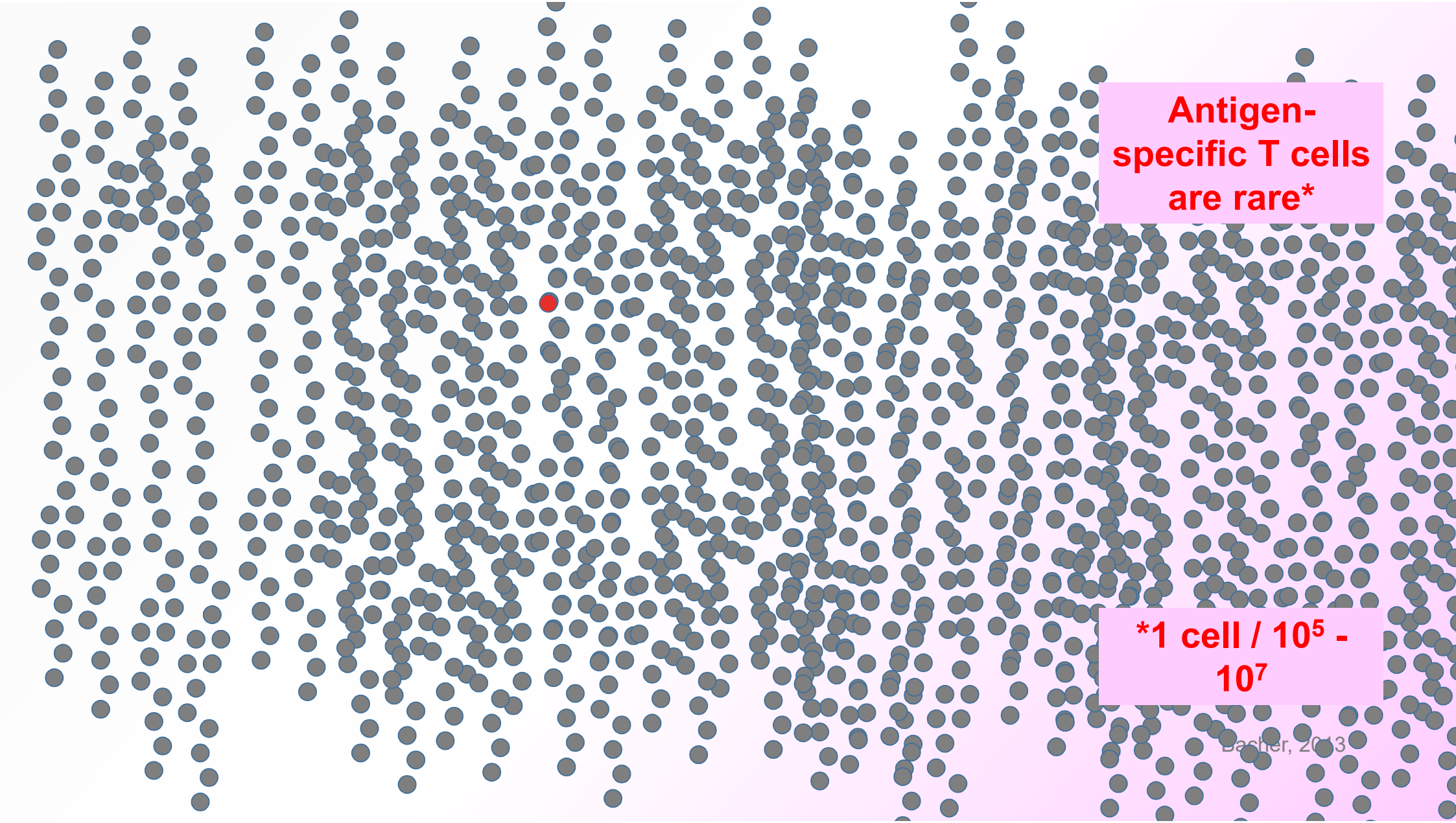
tattoo needle wear in lymph nodes

Schreiver, 2019

Summary part I

- Nanomaterials may sensitize or modulate human immune responses
- Known sensitizer contained in or absored onto nanomaterials include metals, organic sensitizer and allergenic proteins
- Validated approaches to asses nanomaterial toxicity incl. sensitization potency are still under development
- Nanomaterials hardly migrate through intact skin

Part II - T cell activation by sensitizing chemicals


Antigen-specific T cells are rare*
*1 cell / 10^5 - 10^7
Bacher, 2013

How T cells recognize chemical allergnes

- T cells recognize peptides presented by proteins of the major histocompatibility complex (MHC) with weak affinity
- >100 million TCR/indivdiual Robins, 2009

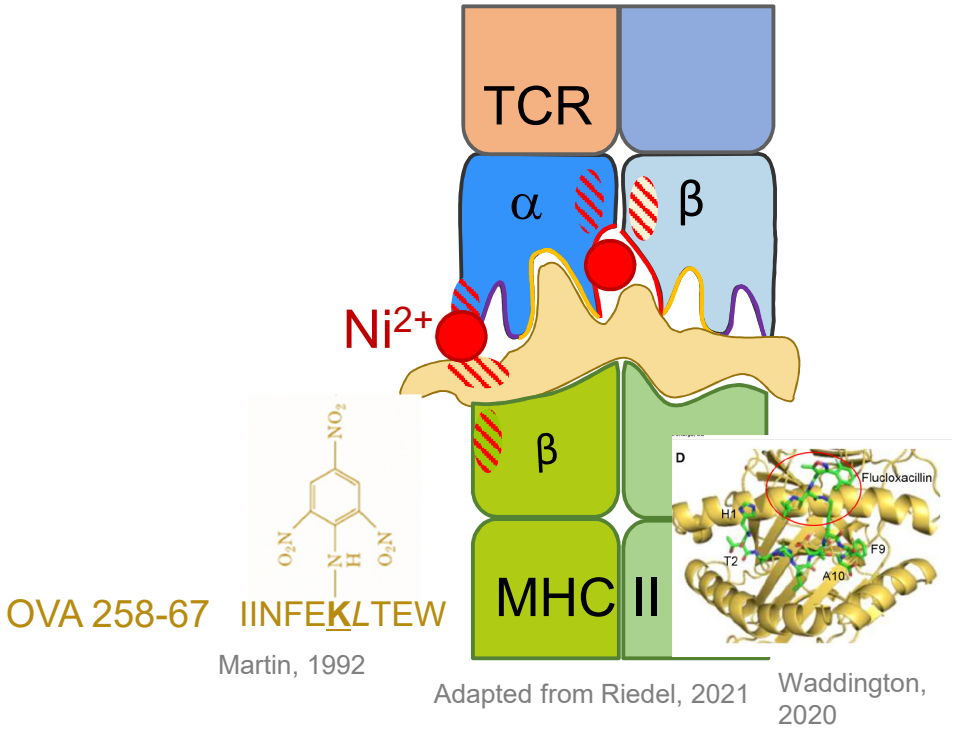
V(D)J-Rekom-bination

CDR1 CDR2 CDR3

V (70/52) N(D)N J (61/13) C

TGTGCTGTGGAATACAAAGCTGCAGGCAACAAGCTAACTTTT

CAVEYKAAGNKLTF



- Haptens bind at the TCR-self-peptide-MHC interface (covalent/ complex formation), → activation threshold exceeded
- T cell activation by 1 ligand possible Sykulev, 1995

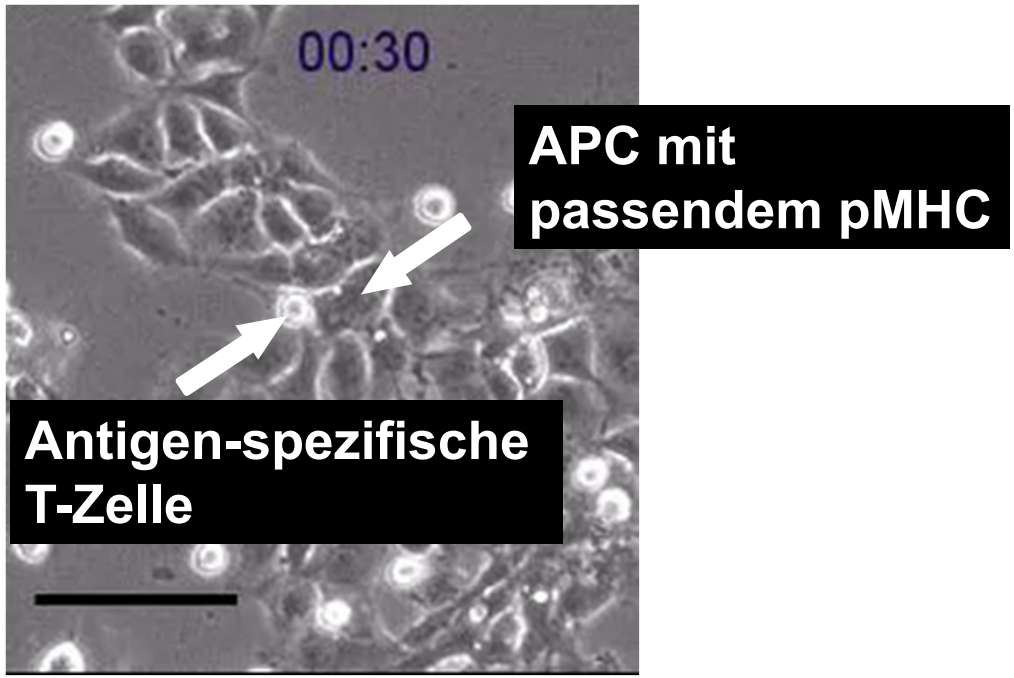
OVA 258-67 IINFE**K***L*TEW

Martin, 1992

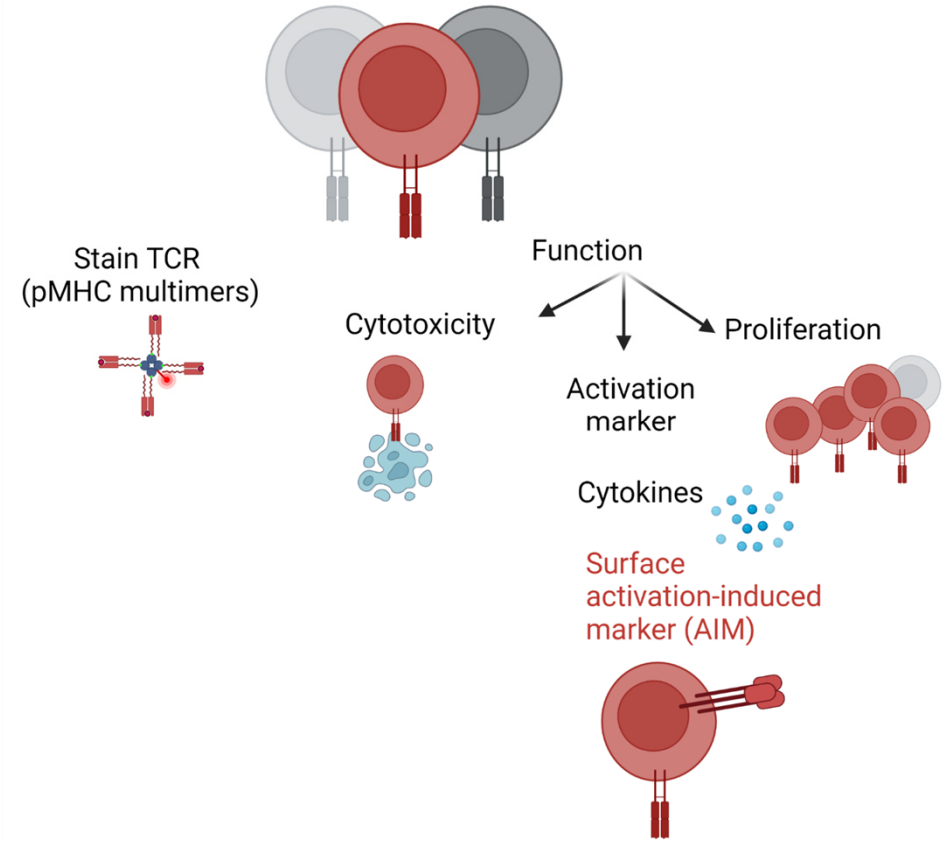
Adapted from Riedel, 2021 Waddington, 2020

CDR – complementarity determining region

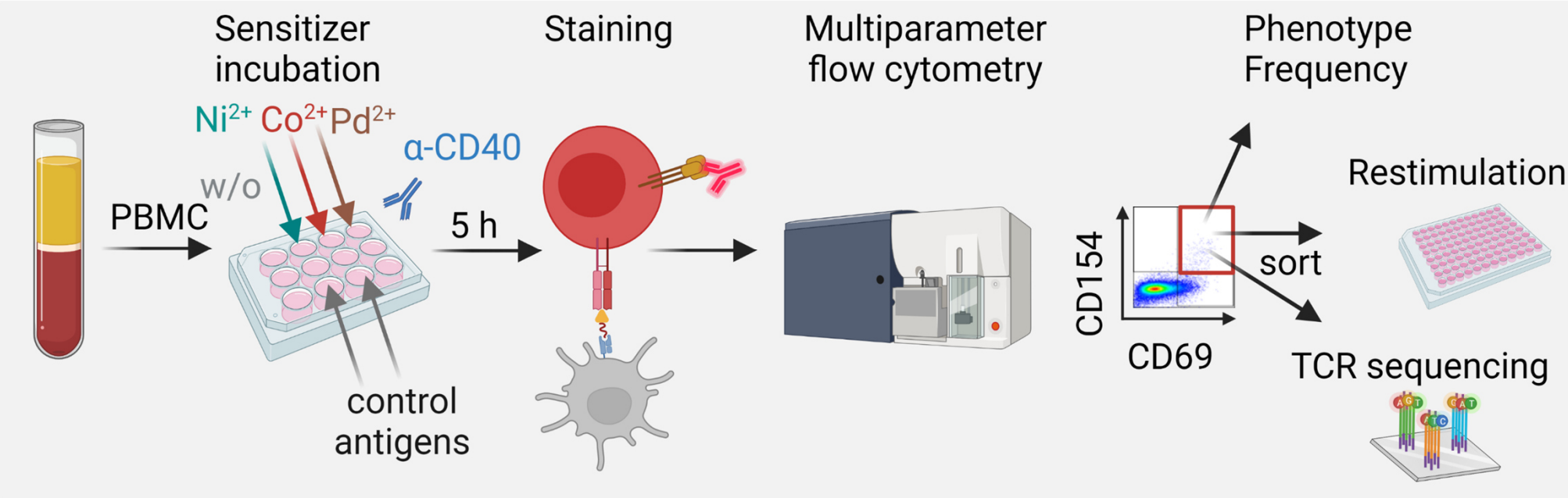
Methods for the detection of rare antigen-specific T cells



Siewert, 2012

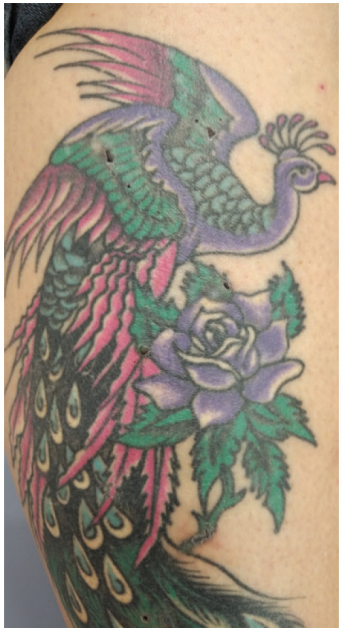


New short-term method: Activation-induced marker (AIM) T cell assay

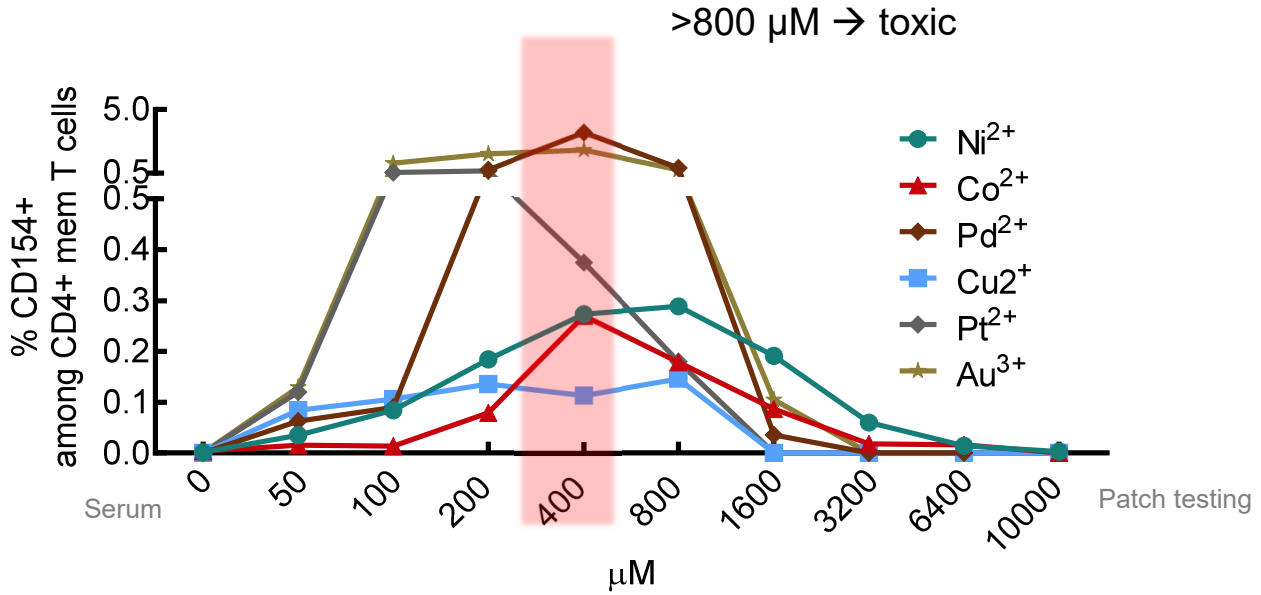


Frentsch 2005, Bacher 2013, Aparicio-Soto 2020, Curato 2022

Metal-specific T cell percentages are concentration dependent



van der Bent, 2019



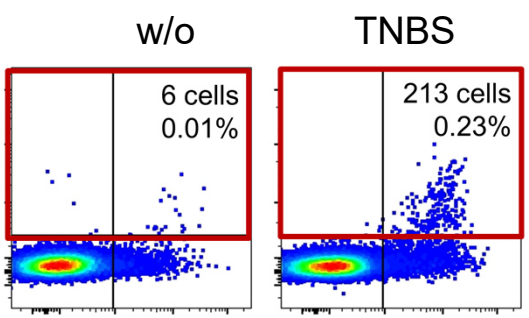
Tattoo allergy from nickel in green tattoo ink

Local in vivo concentrations, e.g. from nanomaterials, remain unknown

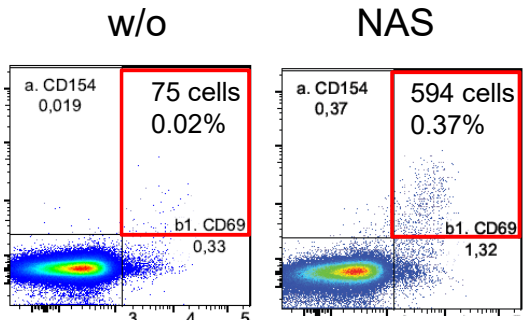
Metal-specific T cells much more frequent than usual protein antigen-specific T cells
Why? → TCR sequencing

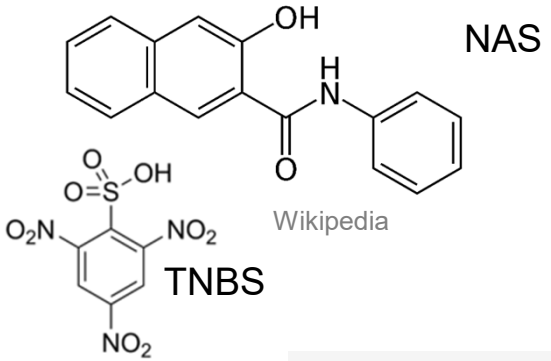
Means, n = 9 (Ni^{2+}, $NiSO_4$), 10 (Co^{2+}, CoCl2), 11 (Pd^{2+}, $PdCl_2$), 4 (Cu^{2+}, $CuSO_4$), 2 (Pt^{2+}, $PtCl_2$) and 4 (Au^{3+}, $HAuCl_4$) buffy coats (likely non-allergic donors).

Adaption of the AIM T cell assay to organic allergenes and tattoo pigments

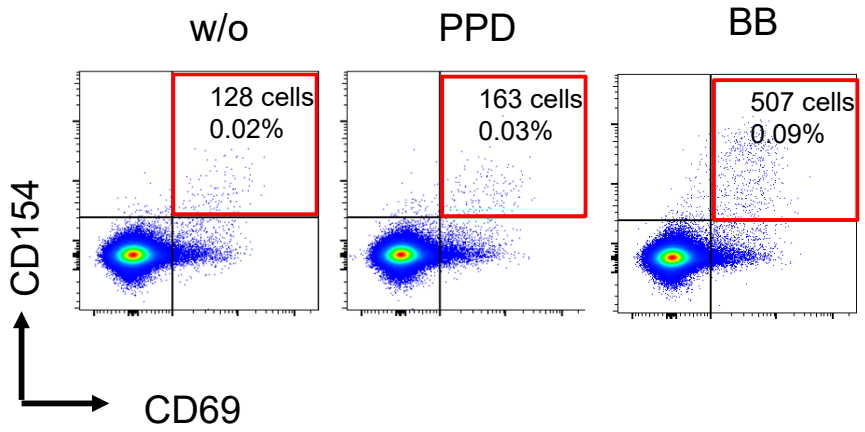


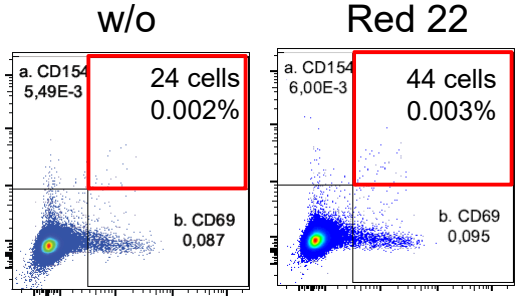
Curato, 2022

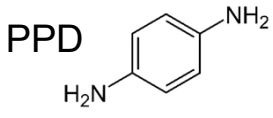


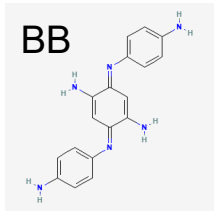


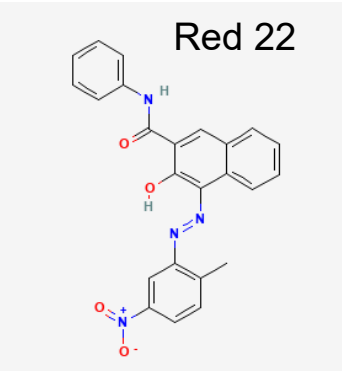
Wikipedia









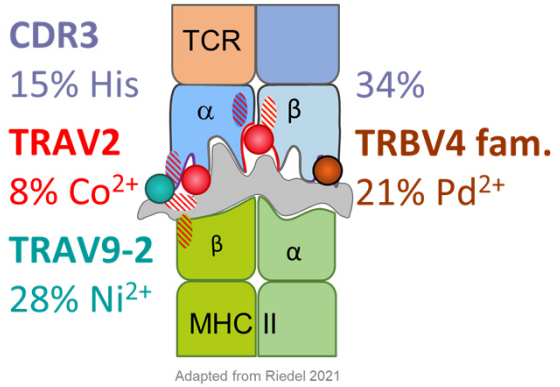


https://pubchem.ncbi.nlm.nih.gov/

w/o – no antigen, TNBS – trinitrobenzenesulfonic acid, NAS – naphthol AS, PPD – p-phenylenediamine, BB - Bandrowsi‘s base

Summary II

- Chemical-specific T cells can be detected by activation-indcued marker (AIM) T cell assays
- Advantages AIM assay: fast, sensitive, quantiative, comprehensive, compatible with live cell isolation
- Metal ion concentrations determine the percentages of activated T cells
- TCR repertoire analysis reveal unusual recognition mechanisms of chemical allergens that underlie T cell activation in non-allergic individuals



Outlook

- Further adaptation of the AIM assay to nanomaterials, further metal allergens (TCR repertoires)
- Optimize epitope generation Nanoparticle encapsulation - Cortial, 2015
- TCR cross-reactivity analysis
- Develop an OECD guidelines for T cell-based assays as part of an IATA
- Further develop *in vitro* allergy diagnosis

IATA – integrated appraoches to testing and assessemnt

GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

20 YEARS Identify Risks – Protect Health

BfR German Federal Institute for Risk Assessment

Thank you for your attention

Katherina.Siewert@bfr.bund.de