Overview of in vitro methodologies for immunotoxicity assessment

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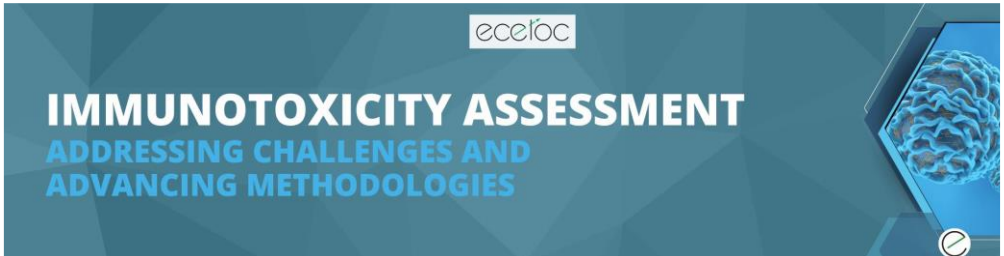


Conflict of Interest Statement

The author declares no conflict of interest.

LAYOUT OF THE PRESENTATION

- Immunotoxicity
- In vitro assessment of immunotoxicity and novel approaches:
  - DRP on in vitro immunotoxicity (immunosuppression)
  - Developmental immunotoxicity
- Conclusions

IMMUNOTOXICITY

DEFINITIONS

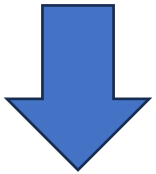
- **IMMUNOTOXICOLOGY** studies the adverse effects of xenobiotics on the immune system.
- **IMMUNOTOXIC COMPOUND** is a compound that can alter one or more immune functions resulting in an **adverse effect** for the host.

A system in Balance

Optimal effectiveness

Immunosuppression

Immune under-reaction



Altered responses to vaccination
Frequent and severe infectious disease
Atypical infections
Cancer

Inappropriate Immunostimulation

Immune over-reaction

Hypersensitivity
Autoimmunity
Chronic inflammation
Unexpected immunostimulation
(e.g., Flu-like syndromes)

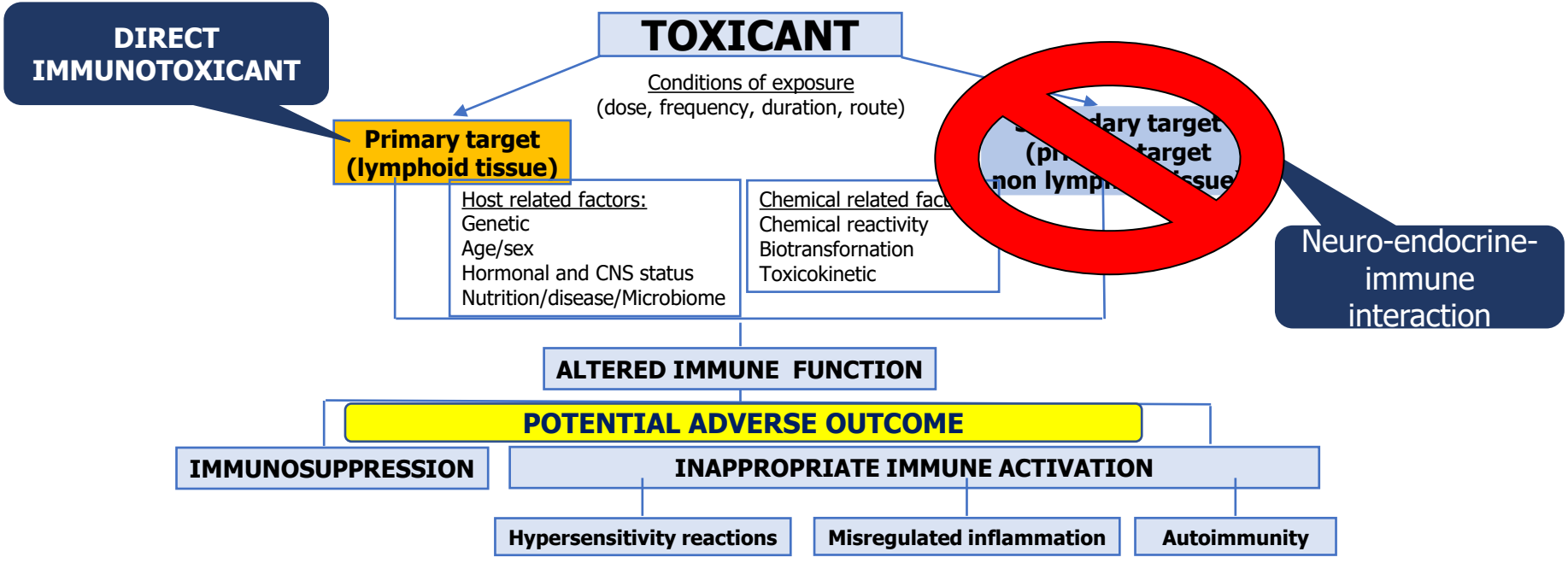
ASSESSMENT OF IMMUNOTOXICITY

- Current assessment of immunotoxicity rely on animal tests, which include some immune endpoints in repeated dose tests and call for dedicated tests only when certain alerts indicate a problem (**case by case**).

- **Different requirements, however, depend on guidelines**, i.e. functional tests are required by US-EPA for pesticides; weight of evidence approach for pharmaceuticals (ICH S8) and chemicals.

How can we study immunosuppression in vitro?

DIRECT IMMUNOTOXICANTS



IN VITRO STUDIES

- A majority of the *in vivo / ex vivo* tests have an *in vitro* counterpart
- *In vitro* studies are often excellent for providing mechanistic or mode of action information
- There have been several successful efforts to validate *in vitro* endpoints with functional immune tests (think of skin sensitization).
- Current in vitro immunotoxicity efforts focus on identifying better biomarkers, improving physiological relevance, and increasing test efficiency.

HOW: NOVEL APPROACHES

- Alongside traditional animal studies, alternative approaches are becoming available.
- The understanding of the Adverse Outcome Pathways underlying immunotoxicity, can support chemical risk assessment based on mechanistic reasoning (OECD, 2020a): five AOPs on immunotoxicity in the OECD work plan are on-going.
- Key characteristics of immunotoxicity have been defined (Germolec et al., 2022).
- Computational models are also available.

COMPUTATIONAL MODELS

- In the field of immunotoxicity, the majority of in silico models have addressed skin sensitization (OECD toolbox, ToxTree, TOPKAT, Derek, TOPS-MODE, etc.).
- There are, however, also in silico programs that address immunosuppression, like ProTox, a freely available webserver for the prediction of toxicity of chemicals, including immunotoxicity. https://tox.charite.de/protox3/index.php?site=compound_input
- The Universal Immune System Simulator (UISS), a state-of-the-art platform for simulating the dynamics of the immune system.

UISS and APPLICATIONS IN TOXICOLOGY

- **Advancing PFAS risk assessment: Integrative approaches using agent-based modelling and physiologically-based kinetic for environmental and health safety.** Comput Struct Biotechnol J. 2024 doi: 10.1016/j.csbj.2024.06.036.
- **Pioneering bioinformatics with agent-based modelling: an innovative protocol to accurately forecast skin or respiratory allergic reactions to chemical sensitizers.** Brief Bioinform. 2024 doi: 10.1093/bib/bbae506.
- **Computational modelling and simulation for immunotoxicity prediction induced by skin sensitisers.** Comput Struct Biotechnol J. 2022. doi: 10.1016/j.csbj.2022.10.032.

How can we study immunosuppression in vitro?

1. OECD and DRP on in vitro immunotoxicity (immunosuppression)

Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)16 | 1

Unclassified

English - Or. English

19 September 2022

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Detailed Review Paper

In vitro tests addressing immunotoxicity with a focus on immunosuppression

Contractor:

Hajime Kojima

Japanese Center for the Validation of Alternative Methods (JaCVAM)

National Institute of Health Sciences

3-25-26, Tonomachi, Kawasaki-ku, Kawasaki, JAPAN

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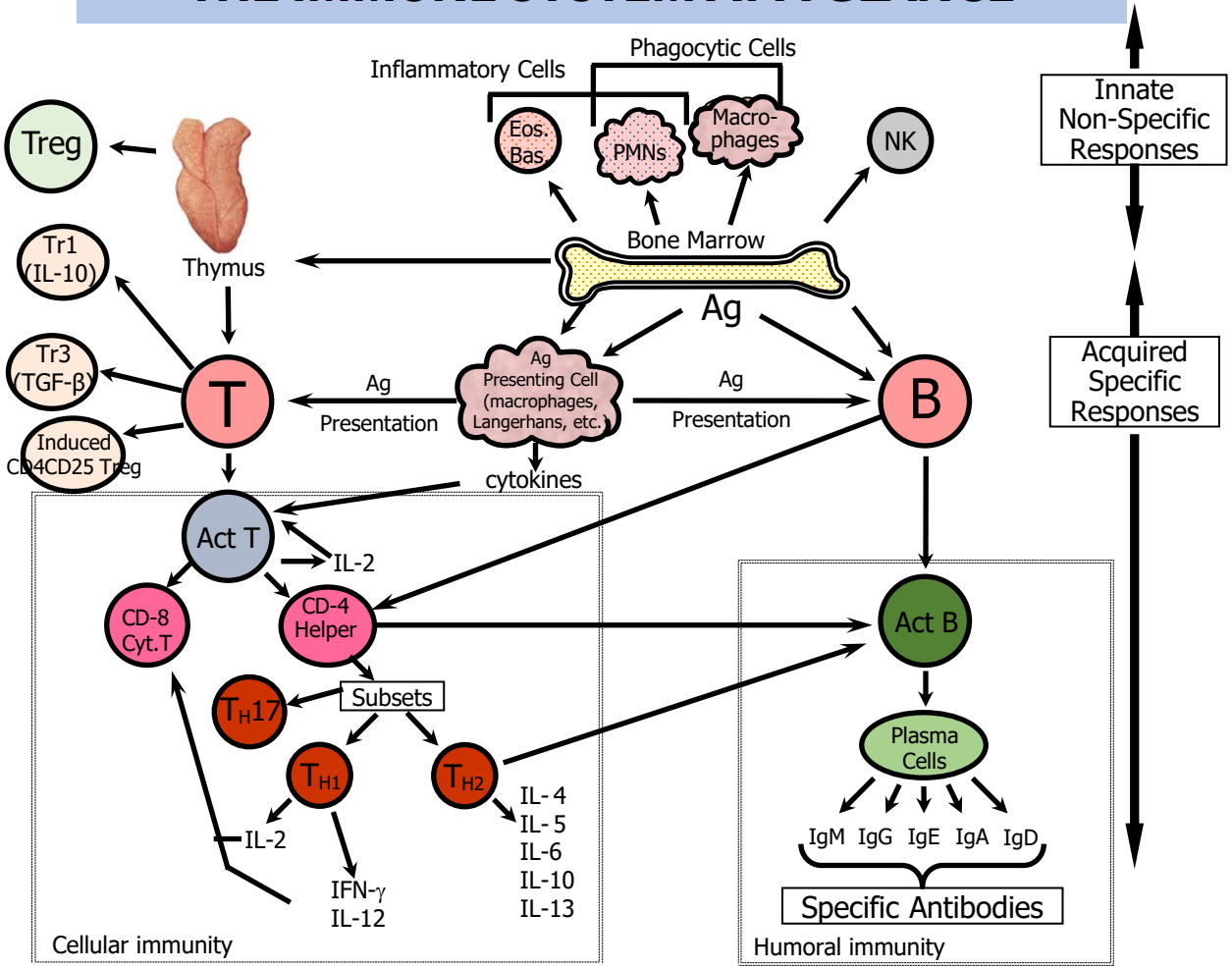
https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/cbc/mono(2022)16&doclanguage=en

THE IMMUNE SYSTEM AT A GLANCE

The immune system is complex, and multiple arms must be considered when evaluating immunotoxicity



Key targets in chemical-induced immunosuppression and in vitro opportunities

- Note: ex-vivo tests to assess immune functions are de facto in vitro methods.

- The CFU-GM, the human whole blood cytokine release assay, and the MITA underwent formal validation.

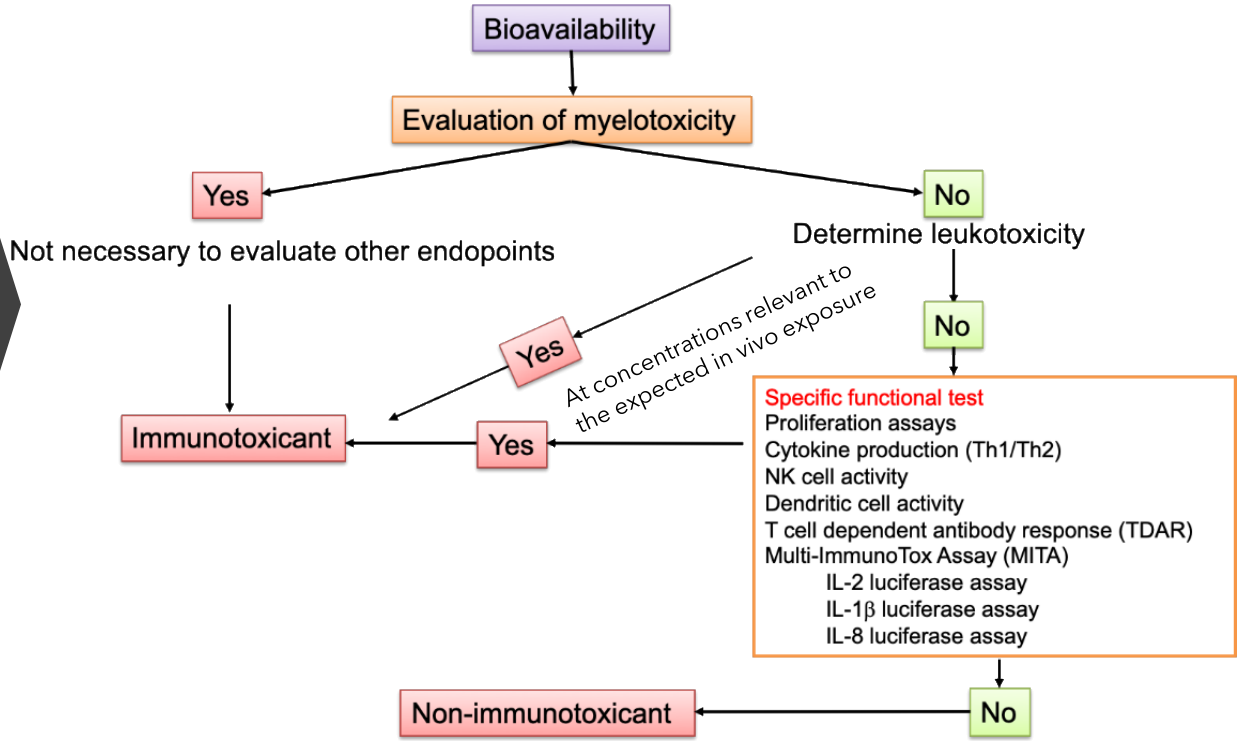
Table 2. Key targets in chemical-induced immunosuppression and *in vitro* opportunities

KEY TARGETS	*IN VITRO* OPPORTUNITIES	CELL MODEL	REFERENCES
Bone marrow	Human lympho-hematopoietic colony-forming assay for myelotoxicity (e.g. CFU-GM)	Human bone marrow and umbilical cord blood; rodent bone marrow	Pessina et al., 2003; 2005; 2010; Rich and Hall, 2005; Haglund et al., 2010
Innate immunity	NK cell activity	Rodent splenocytes; human peripheral blood mononuclear cells	Lebrec at al., 1995
	Monocytes/macrophages cytokines	Human peripheral blood mononuclear cells (e.g. whole blood assay); rodent splenocytes; cell lines (e.g. THP-1)	Langezaal et al., 2001; Langezaal et al., 2002; Carfì et al., 2007; Kimura et al., 2018
Cell mediated immunity	T cell proliferation	Rodent splenocytes; human peripheral blood mononuclear cells	Lebrec at al., 1995; Carfì et al., 2007
	Mixed leukocyte response (MLR)	Rodent splenocytes; human peripheral blood mononuclear cells	Lebrec at al., 1995
	Cytotoxic T lymphocyte (CTL)	Rodent splenocytes; human peripheral blood mononuclear cells	Lebrec at al., 1995
	Cytokine production	Rodent splenocytes; human peripheral blood mononuclear cells (e.g. HWBCRA); human cell lines (e.g. Jurkat T cells)	Langezaal et al., 2001; Langezaal et al., 2002; Ullerås et al., 2005; Carfì et al., 2007; Ringerike et al., 2005; Stølevik et al., 2010; Kimura et al., 2018
	Transcriptomic profiles	human peripheral blood mononuclear cells; human cell lines (e.g. Jurkat T cells)	Hochstenbach et al., 2010; Shao et al., 2014; Schmeits et al., 2015
Humoral immunity	B cell proliferation	Rodent splenocytes; human peripheral blood mononuclear cells	Carfì et al., 2007
	In vitro antibody production	Rodent splenocytes; human peripheral blood mononuclear cells	Koeper et al., 2009; Lu et al., 2009; Collinge et al., 2010; Fischer et al., 2011
	In vitro antigen presentation to T cells	Mouse cell lines (e.g. 3A9; Ch27B)	Lehmann and Williams, 2018

FLOW CHART/DECISION TREE APPROACH TO ASSESS IMMUNOTOXICITY USING IN VITRO METHODS

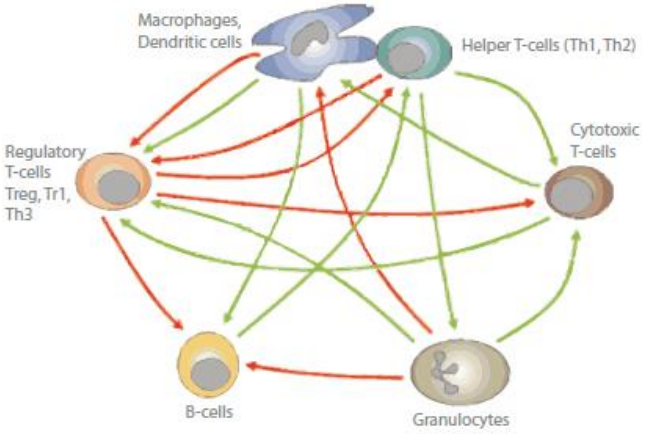
THE TIER APPROACH

By using a tier approach, in vitro methods could provide the means to assess the hazard posed by direct immunotoxicants.



Adapted from Gennari et al, 2005

- Unlike in vitro assays using isolated PBMC, whole blood assays are carried out in the presence of all normal blood components i.e. PBMC, autologous serum, red blood cells, platelets.
- WBA provides a more physiological environment
- WBA is a reliable in vitro method to assess all immune responses, from innate immunity, cell-mediated and humoral.



THE WHOLE BLOOD ASSAY

	Cell Lines	PBMC	Whole Blood
Ease of use / Sourcing	↑↑↑↑	↑	↑↑
Reproducibility	↑↑↑↑	↑↑ Donor variation / processing variation	↑ Donor variation
Complexity	↑ Single cell / not true immune cell / co-culture possible	↑↑ Differential cell type loss due to processing	↑↑↑↑ Most immune cell types present
Human Clinical Relevance	Not directly clinically relevant	↑↑ Moderate relevance	↑↑↑↑ Highly relevant

THE MULTI-IMMUNO-TOX ASSAY

- **MITA**: a high-throughput approach to detect chemical immunotoxicity.
- Stable reporter cell lines (Jurkat T cells, THP-1) transfected with 3 luciferase genes, SLG, SLO, and SLR, under the control of 4 cytokine promoters, IL-2, IFN-γ, IL-1β, and IL-8, and the G3PDH promoter.

Within-laboratory reproducibility: 86.7%.

Between-laboratory reproducibility: 80.0%.

Predictivity: the average predictivity of the Phase I and II studies was 75.0%, while that of additional 60 chemicals examined by the lead laboratory was 82.5%.

ELSEVIER

Toxicology in Vitro
Volume 66, August 2020, 104832

An international validation study of the IL-2 Luc assay for evaluating the potential immunotoxic effects of chemicals on T cells and a proposal for reference data for immunotoxic chemicals

Yutaka Kimura [a], Rie Yasuno [b], Mika Watanabe [c], Miwako Kobayashi [c], Tomoko Iwaki [d], Chizu Fujimura [a], Yoshihiro Ohmiya [b], Kohji Yamakage [c], Yoshihiro Nakajima [d], Mayumi Kobayashi [e], Nana Mashimo [e], Yumi Takagi [e], Takashi Omori [e], Emanuela Corsini [f], Dori Germolec [g], Tomoaki Inoue [h], Erwin L. Rogen [i], Hajime Kojima [j], Setsuya Aiba [a]

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OECD Test No. 444A: In Vitro Immunotoxicity

IL-2 Luc Assay

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Toxicology in Vitro

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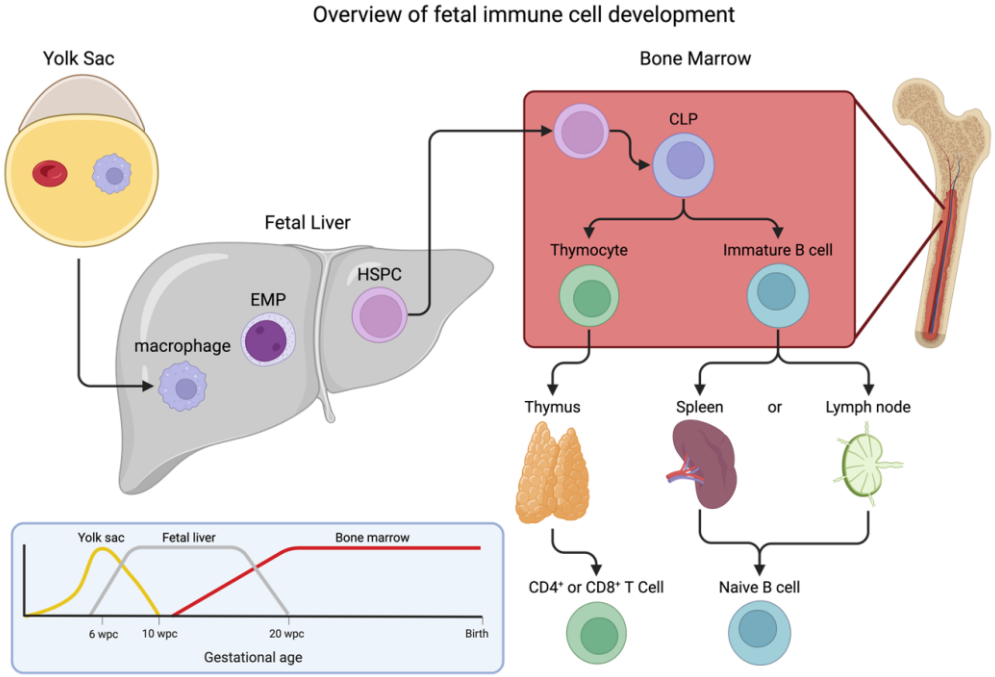
2. Opportunities to study developmental immunotoxicity in vitro



Int. J. Mol. Sci. 2024, 25(11), 5951; https://doi.org/10.3390/ijms25115951

DEVELOPMENTAL IMMUNOTOXICITY IN VITRO

- One possibility will be to use the same assays described before, at least as a screening tool
- Differentiation of dendritic cells and monocytes derived from induced pluripotent stem cells (Park et al., 2024; Senju et al., 2011)
- Differentiation of B cells from human umbilical cord CD34+ cells (Li et al., 2017)
- Differentiation of T cells from human umbilical cord CD34+ cells (Trotman-Grant, 2021)
- Non-mammalian species with intact immune system, such as Zebra fish (by day 5 innate immunity can be addressed)
- Microphysiological system (MPS) models

IMMUNOSUPPRESSION SUMMARY

- Considering the available in vitro methods, it is feasible to explore the immunosuppressive capabilities of chemicals.
- Focus should be on the endpoints reflecting the functional integrity of the immune system.
- Based on the amount of in vivo data that is generally required to define a compound as immunotoxic, it is anticipated that there will be a need for multiple in vitro tests, which should be incorporated into Integrated Approaches to Testing and Assessment (IATA) combinations.
- It is expected that the combined use of several in vitro assays testing different immune targets will increase predictivity over any individual assay alone.
- By using a tier approach, in vitro methods could provide the means to assess the hazard posed by direct immunotoxicants.
- AOPs, key characteristics and computational methods also offer an opportunity to identify immunotoxic compounds.

GAPS

- Further investigation with larger datasets is necessary to distinguish between biomarkers that are representative of immunosuppression in general and biomarkers that reflect a particular type of chemical.
- Different immune cell targets.
- Indirect immunotoxicity (2D, 3D, MPS, cocultures mimicking physiological conditions)
- Better understanding of the mechanisms involved in immunotoxicity to identify a clear toxicological endpoints (multiomics).
- Protocol standardization, high-throughput instruments
- Serum free medium
- International acceptance

I THANK YOU FOR YOUR ATTENTION