



OPEN ACCESS

EDITED BY

Victor J. Johnson,
Burlison Research Technologies, Inc.,
United States

REVIEWED BY

Michael Javadi Nash,
University of Colorado Anschutz Medical
Campus, United States
Yunjia Lai,
Columbia University, United States

*CORRESPONDENCE

Julia Tigges
✉ Julia.tigges@iuf-duesseldorf.de

RECEIVED 07 November 2025

REVISED 20 May 2026

ACCEPTED 19 June 2026

PUBLISHED 09 July 2026

CITATION

Spruck C, Ladeira L, Kuchovska E,
Ostaszewski M, de Caro ER, Corsini E,
Geris L, Staumont B, Fayyaz S, Li Q,
Grimm F, Fritsche E, Koch K and
Tigges J (2026) HIDmap: a physiology-
based map of human immune system
development to support animal-free
research and regulatory innovation.
Front. Immunol. 17:1741650.
doi: 10.3389/fimmu.2026.1741650

COPYRIGHT

© 2026 Spruck, Ladeira, Kuchovska,
Ostaszewski, de Caro, Corsini, Geris,
Staumont, Fayyaz, Li, Grimm, Fritsche,
Koch and Tigges. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License](#)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

HIDmap: a physiology-based map of human immune system development to support animal-free research and regulatory innovation

Christiane Spruck¹, Luiz Ladeira², Eliska Kuchovska¹,
Marek Ostaszewski³, Emilia Rita de Caro¹, Emanuela Corsini⁴,
Liesbet Geris^{2,5,6}, Bernard Staumont², Susann Fayyaz⁷, Qiang Li⁷,
Fabian Grimm⁷, Ellen Fritsche^{8,9,10}, Katharina Koch^{1,8}
and Julia Tigges^{1*}

¹IUF-Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany, ²Biomechanics Research Unit, GIGA Institute, University of Liège, Liège, Belgium, ³Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg, ⁴Department of Pharmacological and Biomolecular Sciences 'Rodolfo Paoletti', Università degli Studi di Milano, Milan, Italy, ⁵Skeletal Biology and Engineering Research Center, Katholieke Universiteit (KU) Leuven, Leuven, Belgium, ⁶Biomechanics Section, Department of Mechanical Engineering, Katholieke Universiteit (KU) Leuven, Leuven, Belgium, ⁷Clariant Produkte (Deutschland) GmbH, Frankfurt am Main, Germany, ⁸DNTOX GmbH, Duesseldorf, Germany, ⁹SCAHT - Swiss Centre for Applied Human Toxicology, Basel, Switzerland, ¹⁰Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

Human immune system development is established through a sequence of tightly regulated processes occurring across multiple embryonic and fetal organs. Despite extensive research, our mechanistic understanding still relies largely on animal models, which often lack translational precision for human biology. This gap is particularly relevant for developmental immunotoxicity (DIT), where early-life exposure to environmental contaminants such as dioxins, PCBs, and PFAS has been associated with long-term immune dysfunction, including reduced vaccine responsiveness in children. To address this need, we created the Human Immune System Development Map (HIDmap), a curated, findability, accessibility, interoperability, and reusability (FAIR)-compliant digital resource that systematically captures prenatal immune ontogeny. The HIDmap integrates literature-derived data into a modular, three-layered structure linking anatomical niches, cell migration, and differentiation trajectories across 24 organs. Implemented in the MINERVA (Molecular Interaction NEtworks VisuAlization) platform, the HIDmap allows interactive exploration of developmental processes and molecular markers relevant to immune function. By embedding the HIDmap within the Adverse Outcome Pathway (AOP) framework, we constructed a putative AOP to demonstrate how the map can help to connect molecular initiating events related to environmental exposures - such as Aryl hydrocarbon receptor (AhR) activation - to downstream immunological key events and adverse outcomes. This resource offers a human-centered foundation for curating and visualizing human developmental immunobiology and may support the future development of predictive

in vitro New Approach Methodologies (NAMs). In the future, the HIDmap may contribute to the identification of critical windows of susceptibility and support the advancement of mechanism-based, animal-free approaches in developmental immunotoxicology.

KEYWORDS

adverse outcome pathway (AOP), developmental immunotoxicity (DIT), human immune system development, new approach methodologies (NAMs), physiological map (PM), systems biology

1 Introduction

The immune system is a complex, distributed network of cells, tissues, and organs that orchestrates the host defense against infections, diseases, and exogenous noxae (2). Multiple organs - including the yolk sac, aorta-gonad-mesonephros (AGM) region, fetal liver, thymus, spleen, and bone marrow - play essential roles in the prenatal development of immune functions (3, 4). Human immune system development unfolds in spatially and temporally coordinated waves. Primitive hematopoiesis begins in the yolk sac around gestational week (GW) 2–3 with the emergence of early hematopoietic cells (5, 6). From GW 4–5 onward, definitive hematopoietic stem cells (HSCs) and multipotent progenitors (MPP) arise in hemogenic regions such as the AGM, marking the onset of the pro-definitive hematopoietic wave (7). These HSCs subsequently migrate to the fetal liver, where they expand and drive hepatic growth (3–5, 7–10), before colonizing the bone marrow, which remains the primary hematopoietic site throughout life (5–10).

To date, most mechanistic insights into immune ontogeny stem from animal models. However, species-specific differences, ethical concerns, and limited translational relevance highlight the need for human-based New Approach Methodologies (NAMs), including *in vitro* and *in silico* systems (11–13). This need is particularly pressing in the context of developmental immunotoxicity (DIT), where current regulatory testing strategies are limited to *in vivo* rodent studies that lack mechanistic resolution. Identifying and protecting critical windows of susceptibility during immune system development is essential to prevent long-term immuno-developmental disorders caused by environmental toxicants. Epidemiological studies demonstrate that environmental toxicants such as dioxins [e.g., 2,3,7,8-Tetrachlorodibenzodioxin (TCDD)], polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFAS) can impair immune system development in humans. Transcriptomic analyses of umbilical cord blood have linked maternal PCB and TCDD exposure to altered gene expression in immune cells and sex-specific epigenetic changes, correlating with reduced vaccine responses in male children at three years of age (14). Moreover, an assessment by the European Food Safety Authority (EFSA), combining human *in vitro* assays and *in silico* models, demonstrated immunotoxic activity of four PFAS (PFOA, PFOS, PFNA, PFHxS) at human-relevant exposure levels, mirroring epidemiological findings of reduced vaccine efficacy (15–19). These findings highlight the need for human-relevant DIT testing strategies to effectively assess the developmental immunotoxic risks of environmental toxicants.

DIT has recently been identified as a Key Area of Regulatory Challenge (KARC) by the European Chemicals Agency (ECHA) in its KARC 2023, 2024, and 2025 documents (20–22), underscoring its relevance for future risk assessment and the transition toward human-relevant safety testing frameworks. Despite this recognition, uncertainties regarding the critical windows of immune system development and associated mechanisms of developmental immunotoxicants remain a prerequisite to the development of targeted testing strategies for DIT using NAMs, particularly in regulatory contexts (23). Such approaches could facilitate the reduction and replacement of animal testing requirements, including in the context of current regulatory information requirements, i.e., DIT cohorts in Extended One-Generation Reproductive Toxicity studies (EOGRS; OECD Test Guideline (TG) 443), in favor of human-relevant and resource-efficient alternatives. These mechanistic gaps can be addressed by integrating experimental data into Adverse Outcome Pathways (AOPs) (24, 25). At present, there is no single dedicated DIT-specific AOP, although lessons from the field of developmental neurotoxicity (DNT) demonstrate how mechanistic knowledge gaps can be addressed using AOPs and NAMs. First, DNT AOPs (26, 27) link standard approaches and NAMs and support mechanism-based next generation risk assessment (NGRA) (28), particularly when embedded in integrated approaches to testing and assessment (IATA) (29). Second, the establishment of the DNT *in vitro* battery (DNT IVB) provides a regulatory-relevant example of how key developmental processes can be mechanistically mapped and translated into NAM-based risk assessment (15, 30–35). A similar conceptual framework could accelerate the development of predictive, standardized approaches for DIT.

To achieve this, a structured, physiology-based overview of prenatal immune system development is essential. Physiological maps (PM) provide standardized, expert-curated models of biological processes and offer an integrative framework for mechanistic understanding across disciplines (36–40). They can serve as a reference for basic immunology, computational modeling, test method and AOP development and further toxicological applications (36).

Here, we present the Human Immune System Development Map (HIDmap; <https://disease-maps.io/immunedev/>) offering a visual modular and computational representation of prenatal human immune system development relevant for research and regulatory applications.

2 Materials and methods

2.1 Literature search and selection

The initial foundation of the HIDmap was established using nine studies selected based on expert judgement and their relevance to human immune system development (7, 41–48). To expand the HIDmap and address remaining gaps, a structured literature search was conducted in PubMed on September 23, 2022, using the following query:

(Immune system AND development AND hematopoiesis) NOT (cancer) NOT (gut) NOT (breast feeding) NOT (atherosclerosis) NOT (covid-19) NOT (brain).

Only publications from the preceding five years (September 2017 to September 2022) were considered. This timeframe was selected to prioritize contemporary high-resolution datasets relevant to human immune development while maintaining a manageable scope for the initial HIDmap version. The search yielded 284 publications, which were imported to Sysrev, an online platform supporting systematic literature review through customizable labeling and filtering criteria (49).

A two-step screening process was applied: (i) title and abstract screening using the exclusion criteria “absence of human data”, “no relevance to immune system development”, “wrong window of development” (excluding postnatal data), and “focus on diseases”, and (ii) full-text screening applying the same criteria together with the additional criterion: “full text not available”. Following this process, twelve additional publications were identified as primary sources for HIDmap construction (50–61). The full selection process is outlined in Figure 1. The HIDmap is based exclusively on peer-reviewed publications. In total, 21 publications were included, of which 10 used single-cell RNA-seq data (42, 46, 47,

50, 54, 56, 58–61), thereby providing a high level of molecular detail. Multiple data modalities were integrated into the HIDmap, including single-cell datasets, histological findings, and flow cytometry data. At this exploratory stage of HIDmap development, no formal evidence-weighting strategy was applied across different data modalities.

2.2 Network curation

Biochemical and gene-regulatory networks for the HIDmap were constructed using CellDesigner (version 4.4.2), a process diagram editor based on the Systems Biology Markup Language (SBML) (62, 63) and the Systems Biology Graphical Notation (SBGN) standards (64, 65). The activity flow notation of SBGN was adapted (Figure 2) and employed, as it emphasizes biological functions while maintaining a simplified representation suitable for high-level interpretation (64–66). Each arrow was manually curated and annotated with relevant literature using PubMed Identifier (PMID), following the Minimal Information Requested in the Annotation of Models (MIRIAM) guidelines (67). All elements were encoded in a standardized format to support transparency and traceability.

2.3 Resource architecture

The HIDmap is organized into three hierarchical layers of biological granularity (Figure 3): The top layer provides an anatomical overview of all included organs, summarizing the spatial contextualization of human immune system development. Clicking on a specific organ directs the user to the corresponding detailed submap. Embedded in the overview layer are two buttons: One leads to a separate figure of “prenatal timing of immune

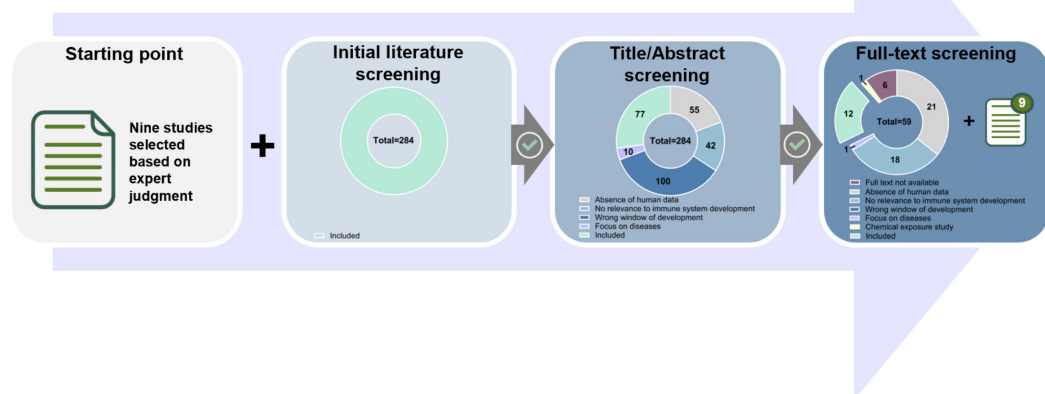


FIGURE 1

Workflow for literature screening with Sysrev to identify studies on human immune system development. The process began with the manual inclusion of nine studies based on expert judgment. An additional 284 studies were identified through a PubMed search (as of September 23, 2022) and subjected to three sequential screening steps: initial literature screening, title/abstract screening, and full-text screening. Following title and abstract review, 59 studies were retained. Of these, 12 met the inclusion criteria after full-text evaluation. Studies were excluded at each stage based on the following criteria: no relevance to immune system development, focus on disease outcomes, chemical exposure studies, or lack of human data. Donut charts indicate the number of included (green) and excluded studies at each step.

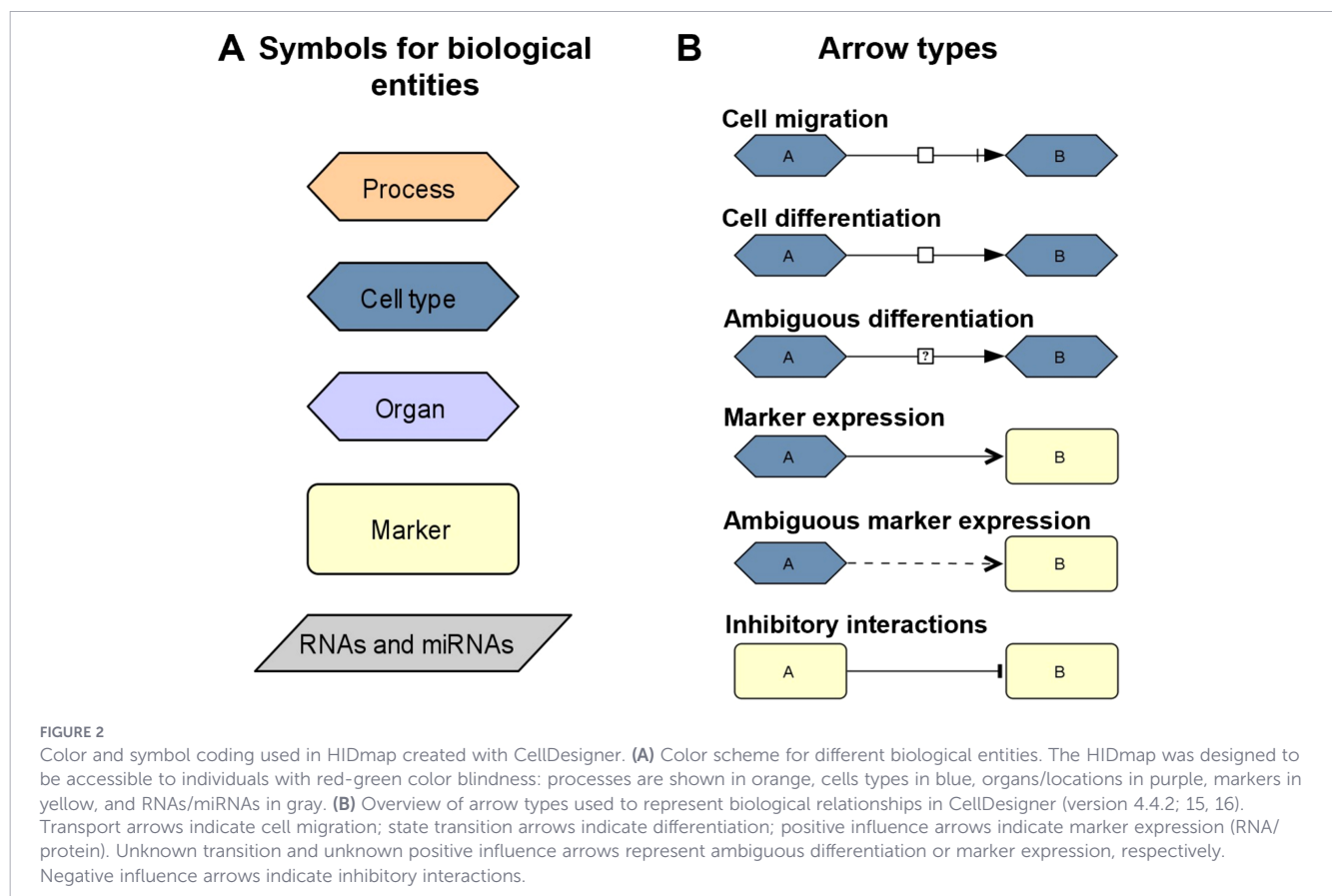
development”, and the “cell migration” button links to the second layer of the map, visualizing dynamic migration of immune cells between organs such as fetal liver, thymus, and bone marrow. Schematic illustrations of organs were created using BioRender (BioRender.com) and incorporated as background elements for anatomical orientation. This layer highlights the spatial complexity of immune cell movement. The third and most detailed layer of cell differentiation can be accessed via the overview layer and features 24 organ-specific submaps. These depict immune cell differentiation pathways and include cell type-specific expression profiles of genes and proteins (markers) as annotations. Migration destinations of individual cell types are also shown, with interactive navigation links enabling navigation between organ maps. This modular framework facilitates structured development, visualization, and iterative refinement of the HIDmap. As part of the Disease Maps Community, long-term maintenance and sustainability of the resource are supported through community engagement via the Molecular Interaction Networks Visualization (MINERVA) platform, including commenting, feedback, and review of future map updates. An informative summary of the project can be found at <https://disease-maps.io/immunedevel/>.

2.4 Data visualization

The HIDmap was deployed using the MINERVA platform, a web-based platform for the curation, annotation and visualization

of molecular interaction networks in Systems Biology Graphical Notation (SBGN)-compatible format (63, 68). Network files created in CellDesigner were merged and uploaded to MINERVA to enable interactive navigation, standardized visual representation, and modification of the HIDmap within a user-friendly, browser-accessible environment. A detailed user guide describing navigation and functionality within the MINERVA platform is provided as [Supplementary Material](#) (Supplementary Material “HIDmap user guide”). Additional examples and tutorials are available through the Inflammatory Skin Disease map (69), <https://disease-maps.io/isdguide/>, and the EU Horizon Europe 2020 “Ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next generation risk assessment (ONTOX)” project (37) resources (<https://ontox-project.eu/physiological-maps-2/>).

To support accessibility, a color scheme optimized for red-green color vision deficiency was applied (e.g., processes: orange; cell types: blue; markers: yellow; Figure 2A). Additionally, CellDesigner’s arrow types were adapted to distinguish different biological relationships within the HIDmap (Figure 2B), including transport arrows for cell migration, state transition arrows for differentiation, positive influence arrows for marker expression, unknown transition arrows for ambiguous differentiation processes, unknown positive influence arrows for ambiguous differentiation or expression, and negative influence arrows for inhibitory interactions. The nomenclature has been standardized using symbols approved by the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC) (70).



2.5 Development of a putative DIT AOP

A putative AOP centered on aryl hydrocarbon receptor (AhR) activation was developed to conceptualize potential mechanisms of DIT. The AOP was informed by available scientific evidence and expert biological knowledge. Although it was not derived from a formal systematic literature review or fully aligned with the OECD “Guidance Document for developing and assessing Adverse Outcome Pathways (71)”, it represents a plausible framework intended to support hypothesis generation and to demonstrate how the HIDmap can be used to identify, contextualize, and organize key events (KE) within an AOP framework.

3 Results and discussion

3.1 Overview of HIDmap architecture and content

We developed the HIDmap, a publicly available, findability, accessibility, interoperability, and reusability (FAIR)-compliant resource that systematically visualizes prenatal human immune system development in a structured and interactive format (72). The HIDmap integrates curated literature data into a repository of systems biology diagrams and depicts immune cell differentiation and migration across embryonic and fetal immune system-related organs, including the AGM region, yolk sac, fetal liver, spleen, thymus, bone marrow, and peripheral organs such as the thyroid primordium, kidney, heart, lung, pleural cavity, lymph nodes, brain, parotid gland, placenta, blood, pancreas, skin, omentum, lamina propria, Peyer’s patches, ileum, duodenum and intestine (Figure 3). Within and between these tissues, the HIDmap tracks key immune cell populations such as HSCs, progenitor cells, T-cells, B-cells, dendritic cells, macrophages, and monocytes.

The HIDmap follows a three-layered architecture (Figure 3A) supporting spatial contextualization of immune ontogeny. The overview layer visualizes involved organs, the migration layer illustrates developmental cell migration, and the cell differentiation layer comprises 24 organ-specific submaps detailing lineage trajectories and cell type-specific marker expression profiles. In addition, a general overview of the timing is provided (Figure 3B). The HIDmap is publicly accessible via the MINERVA platform at <https://immunedeveluxembourg.org/minerva/> (Figure 4).

In total, the HIDmap contains 560 cell types and 185 migration events. Annotation depth varies across 191 organs (Figure 5A; Supplementary Table 1), with the fetal liver (125 cell types), thymus (105), and the 192 yolk sac (75), representing the most comprehensively annotated developmental compartments. By contrast, organs such as the pancreas or duodenum currently contain fewer annotated cell types (one and three, respectively), reflecting limited data availability and/or reduced representation in the literature. Migration analyses further characterized inter-organ migration

relationships within the HIDmap (Figures 5B, C; Supplementary Table 2). The migration landscape analysis (Figure 5B) demonstrated distinct organ-specific migration profiles, with the fetal liver and AGM region showing predominantly outgoing migration patterns consistent with their roles as major developmental source compartments, whereas the thymus displayed a comparatively incoming-dominant profile. In contrast, organs such as the pancreas, thyroid primordium, and heart exhibited only a few migration events and clustered close to the origin of the migration landscape. Net migration polarity analysis (Figure 5C) further highlighted these differences by summarizing the balance between outgoing and incoming migration events across organs. However, these analyses reflect the current structural representation of migration relationships within the HIDmap rather than temporally resolved developmental dynamics, which remain limited by the underlying map architecture. A representative fetal liver submap is shown in Figure 6, illustrating cell populations and their associated marker profiles. All markers are systematically annotated using official HGNC symbols (Figure 7A), while non-standard markers (e.g., major histocompatibility complex (MHC) class II) are displayed in yellow boxes (Figure 7B) to support transparency and traceability of molecular annotations. All entries are manually curated and linked to PubMed references.

The HIDmap is designed as an interactive and continuously evolving resource. This “living” character is supported by the MINERVA Platform, which is a FAIR service (1) that enables community-driven annotation, feedback, and content refinement (68). Users can attach comments directly to specific molecular interactions or entities and upload custom data overlays to contextualize experimental datasets within the map (63). Community annotations undergo curator review before integration to maintain scientific integrity and technical standardization. In addition, MINERVA supports annotation verification against standard databases including PubMed, UniProt, and ChEBI. Tutorials and additional documentation for MINERVA are available online (<https://minerva.pages.uni.lu/>).

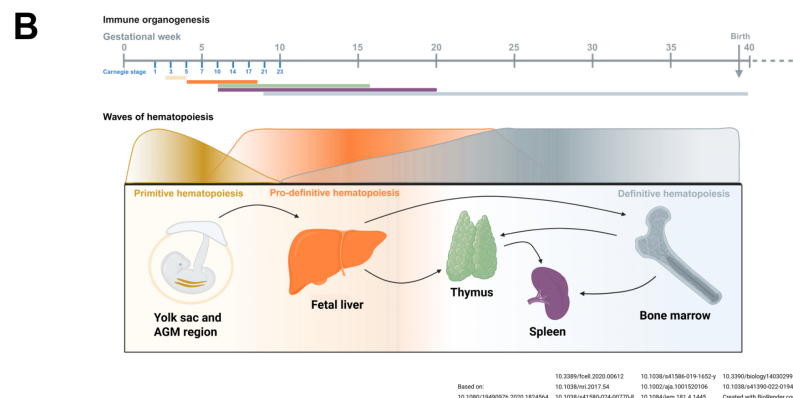
Furthermore, community-driven projects such as the Parkinson’s Disease Map (74) and the COVID-19 Disease Map (73) illustrate the utility of centralized disease maps for collaborative curation and representation of complex biological mechanisms (39).

3.2 Integration of the HIDmap into AOP and NAM frameworks

A central application of the HIDmap is the integration of developmental immune processes into adverse outcome pathways (AOP). AOPs provide structured representations linking molecular initiating events (MIEs), key events (KEs), and adverse outcomes (AO), whereas physiological maps (PMs) contribute mechanistic and spatial context across molecular, cellular, and tissue levels. Together, both approaches support the organization and interpretation of mechanistic knowledge relevant to DIT and NAM development. This concept has been exemplified for cytokine release syndrome (CRS), where physiological maps were used to expand

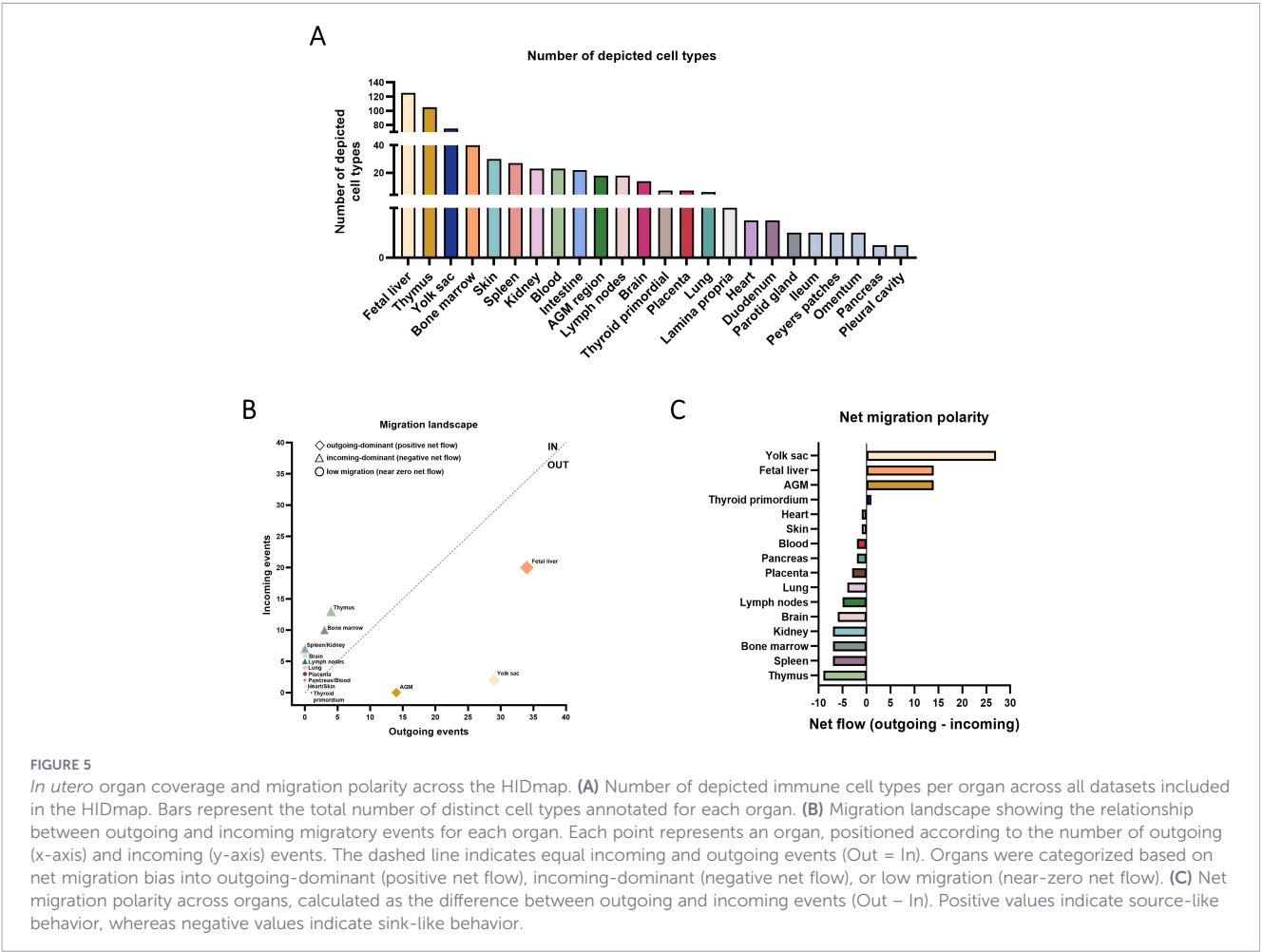
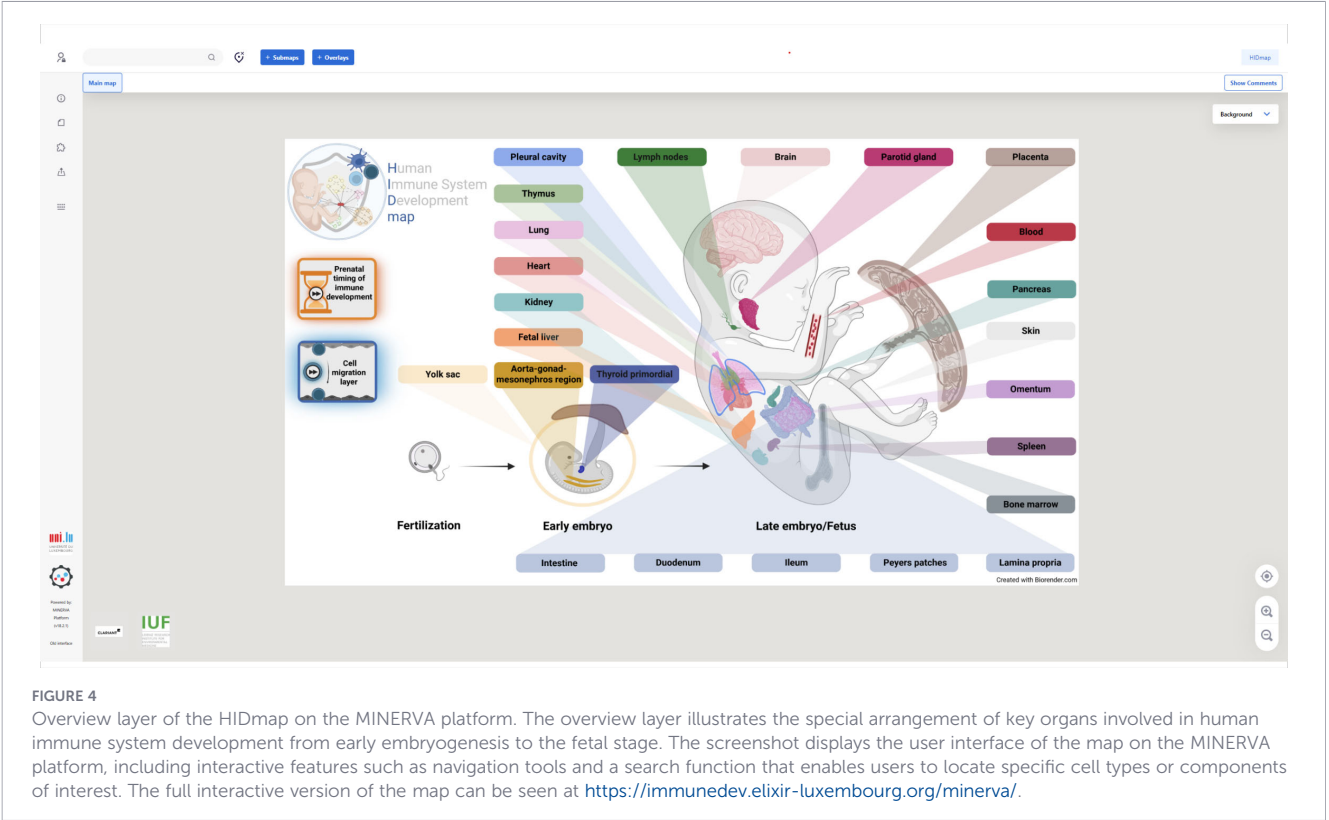
HIDmap as a complementary framework for organizing mechanistic information related to prenatal immune system perturbations. To illustrate this concept, we developed a putative AOP involving activation of the AhR during critical windows of immune development. In this framework, AhR activation (MIE) is linked to impaired HSC proliferation (KE1) (78–80), reduced lymphoid hematopoiesis (KE2) (81), diminished T- and B-cell development and peripheral pools (KE3-KE4) (78, 81–85), and in parallel, impaired antigen-presenting cell maturation (KE5) (82, 83, 86, 87). These upstream perturbations result in thymic atrophy (KE6) (85, 88, 89), disrupted lymph node architecture (KE7) (89), and attenuated T- and B-cell dependent immune responses (KE8) (90–

HIDmap as a complementary framework for organizing mechanistic information related to prenatal immune system perturbations. To illustrate this concept, we developed a putative AOP involving activation of the AhR during critical windows of immune development. In this framework, AhR activation (MIE) is linked to impaired HSC proliferation (KE1) (78–80), reduced lymphoid hematopoiesis (KE2) (81), diminished T- and B-cell development and peripheral pools (KE3-KE4) (78, 81–85), and in parallel, impaired antigen-presenting cell maturation (KE5) (82, 83, 86, 87). These upstream perturbations result in thymic atrophy (KE6) (85, 88, 89), disrupted lymph node architecture (KE7) (89), and attenuated T- and B-cell dependent immune responses (KE8) (90–



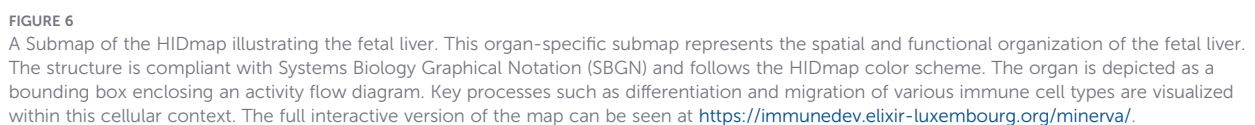
Prenatal development of the human immune system

Structure of the human immune system development map (HIDmap). **(A)** The HIDmap is organized into three hierarchical levels. The first (overview) displays anatomical structures and organs involved in immune system development. The second (migration) depicts the spatial migration patterns of immune cell types between organs. The third level comprises 24 organ-specific submaps, illustrating detailed information on cell differentiation and marker expression, including protein and gene expression. The HIDmap is entirely based on human data. **(B)** The figure illustrates prenatal immune system development, showing immune organogenesis and waves of hematopoiesis. Gestational weeks and Carnegie stages are displayed as a timeline at the top. Below, organogenesis is depicted as color-coded bars (yolk sac: yellow; fetal liver: orange; thymus: green; spleen: purple; bone marrow: gray) with the three waves of hematopoiesis shown at the bottom.



niches and processes (Figure 9B). By contrast, downstream organism-level responses (e.g., KE8 and AO) remain beyond the current scope of the HIDmap, reflecting its primary utility for upstream and cell-based key events. Thus, the HIDmap is best viewed as a structured, mechanistic framework supporting AOP development and refinement. In addition, the HIDmap may inform future NAM development by providing structured insights into developmental processes, cell migration patterns, and cell-type-specific marker expression profiles that could serve as candidate endpoints for DIT testing strategies. These curated developmental processes may guide future efforts toward the establishment of human-relevant *in vitro* test methods for assessing the DIT potential of

DIT testing strategies. These curated developmental processes may guide future efforts toward the establishment of human-relevant *in vitro* test methods for assessing the DIT potential of



environmental toxicants. However, additional validation, benchmarking against existing approaches, and demonstration of fitness-for-purpose will be required before regulatory application can be considered.

Lessons learned from developmental neurotoxicity (DNT) research further demonstrate how mechanistically anchored complex developmental processes can be translated into predictive *in vitro* testing strategies (30, 31, 34, 72). A similar framework for DIT, anchored in AOPs and supported by physiologically informed resources such as the HIDmap, may provide a basis for future development of predictive *in vitro* DIT testing approaches.

3.3 The HIDmap in the context of existing bioinformatic resources

Platforms such as Kyoto Encyclopedia of Genes and Genomes [KEGG (95)], Reactome (96), and WikiPathways (97) provide well-curated pathway representations, but do not specifically capture the

context of human immune system development *in utero*. The HIDmap addresses this gap by providing an integrated, tissue-specific overview of prenatal immune system development. While these resources provide greater detail for individual pathways, such depth is beyond the scope of the current HIDmap version. Instead, the HIDmap is intended as a complementary resource providing an accessible overview of organs, cell types, markers, and key developmental processes including migration and differentiation.

Ingenuity Pathway Analysis (IPA) is a widely used commercial platform for literature-based omics interpretation and mechanistic hypothesis generation (98). However, IPA relies on generalized literature-derived networks and automated curation approaches, which may bias analyses toward well-studied pathways. In addition, IPA does not provide a developmental framework specific to prenatal immune system development and remains commercially restricted. Consequently, IPA complements rather than replaces manually curated developmental frameworks such as the HIDmap.

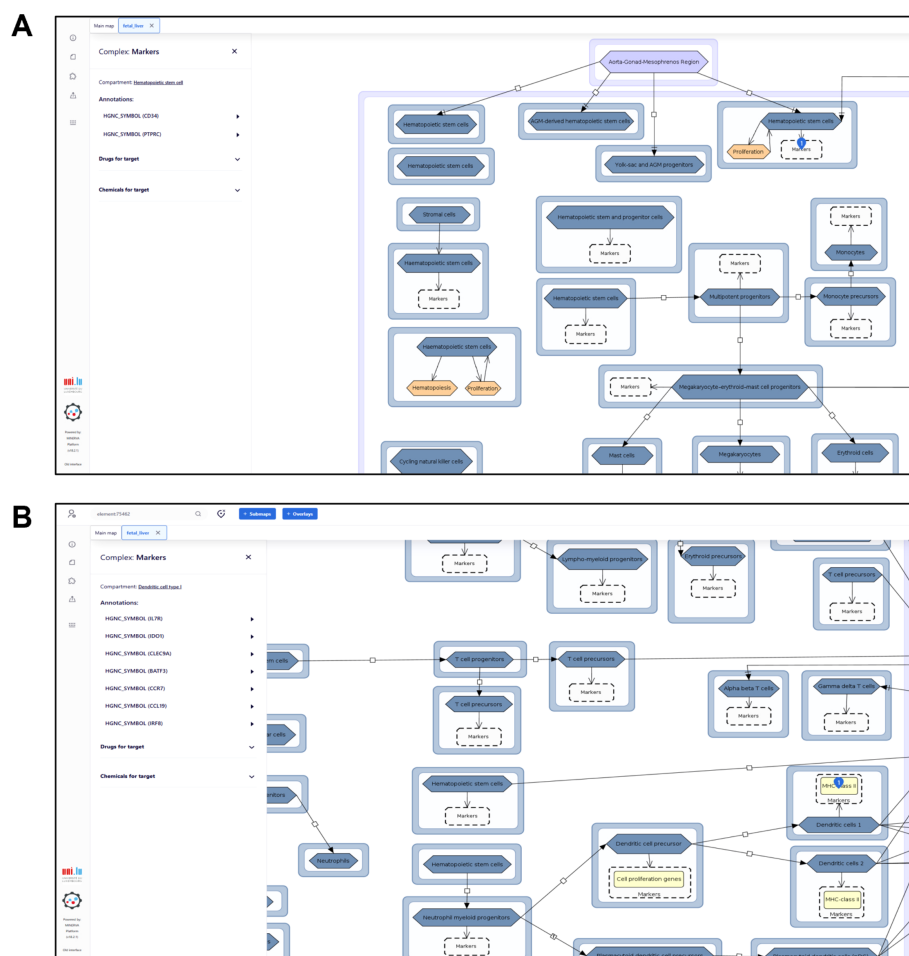


FIGURE 7

Visualization of marker expression of the different immune cells on the MINERVA platform. Standard representation of marker annotations using the official HGNC symbols are shown on the left. (A) Example of hematopoietic stem cell markers. (B) Marker annotation exceptions: yellow boxes indicate markers without an official HGNC symbol (e.g., MHC class II); this information is retained within the yellow box. Markers with official HGNC symbols remain accessible on the left after clicking on box. The full interactive version of the map can be seen at <https://immunedeveluxembourg.org/minerva/>.

The Human Cell Atlas (HCA) provides high-resolution single-cell and spatial transcriptomic datasets (99), that have substantially advanced the characterization of fetal immune development and hematopoiesis. However, HCA primarily functions as a data repository rather than an integrated developmental framework and typically requires substantial bioinformatic expertise for data processing and interpretation. Consequently, HCA complements rather than replaces curated overview resources such as the HIDmap. Direct comparison of HIDmap and HCA annotations demonstrated overall concordance across major hematopoietic lineages while highlighting differences in developmental resolution. The HIDmap retained embryonic and yolk-sac-specific intermediates such as erythro-myeloid progenitors (EMP) and yolk sac-derived primitive macrophages, whereas corresponding HCA annotations were often represented by broader hematopoietic or myeloid labels. Conversely, HCA included several adult-like immune annotations that may reflect reference-atlas-driven annotation transfer rather than bona fide yolk-sac developmental states (Supplementary Figure 1). Notably, both resources include the yolk

sac dataset published by Suo et al., 2022 (47), although the HIDmap additionally integrates information from further studies. Together, these findings suggest that the HIDmap complements HCA by preserving transient embryonic and ontogenetic information that may become obscured in broader reference-based annotation frameworks.

Taken together, these comparisons highlight the role of the HIDmap as a complementary resource integrating developmental and tissue-specific information related to prenatal immune system development. The HIDmap combines literature-derived omics, flow cytometry, and histological data into a structured and accessible framework that supports exploration of developmental markers, cell types, migration, and differentiation processes. Importantly, the HIDmap consolidates currently fragmented data on *in utero* immune system development by consolidating available knowledge onto a single platform. Unlike pathway-centered resources such as Reactome and KEGG, the HIDmap starts from developmental context and links it to relevant biological migration and differentiation processes.

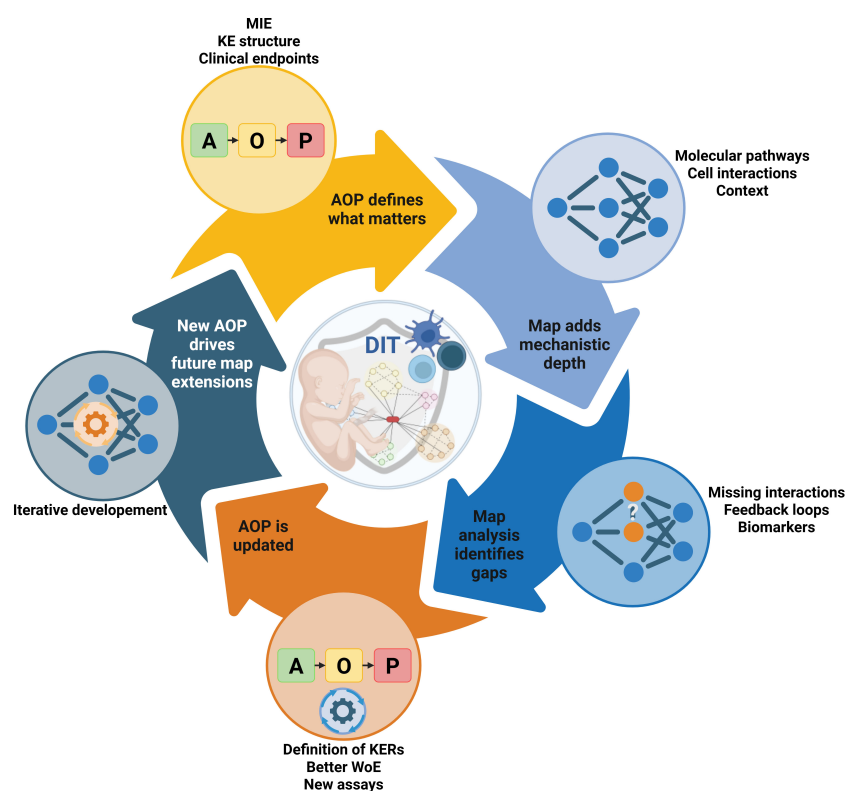


FIGURE 8

Conceptual framework for the iterative and bidirectional integration of the Human Immune System Development Map (HIDmap) and adverse outcome pathways (AOPs). The diagram illustrates how physiological maps (PMs) and AOPs work together in a complementary manner to improve our mechanistic understanding of developmental immunotoxicity (DIT). The HIDmap contributes to advancing mechanistic understanding by embedding key events (KE) in their molecular, cellular, and tissue-related context during human prenatal immune development. Subsequent analysis of the map enables the systematic identification of knowledge gaps, inconsistencies, and missing connections, which serve as the basis for refining existing KEs and adding new ones within an AOP. The revised AOP, then, guides the further expansion and curation of the PM. The updated map will then provide an enriched framework of toxicologically relevant biology for subsequent AOP iterations, thereby closing a continuous feedback loop. Through this cycle, the HIDmap and AOPs evolve together to support hypothesis-driven AOP development and to structure mechanistic representations of prenatal immune system disruptions relevant to DIT.

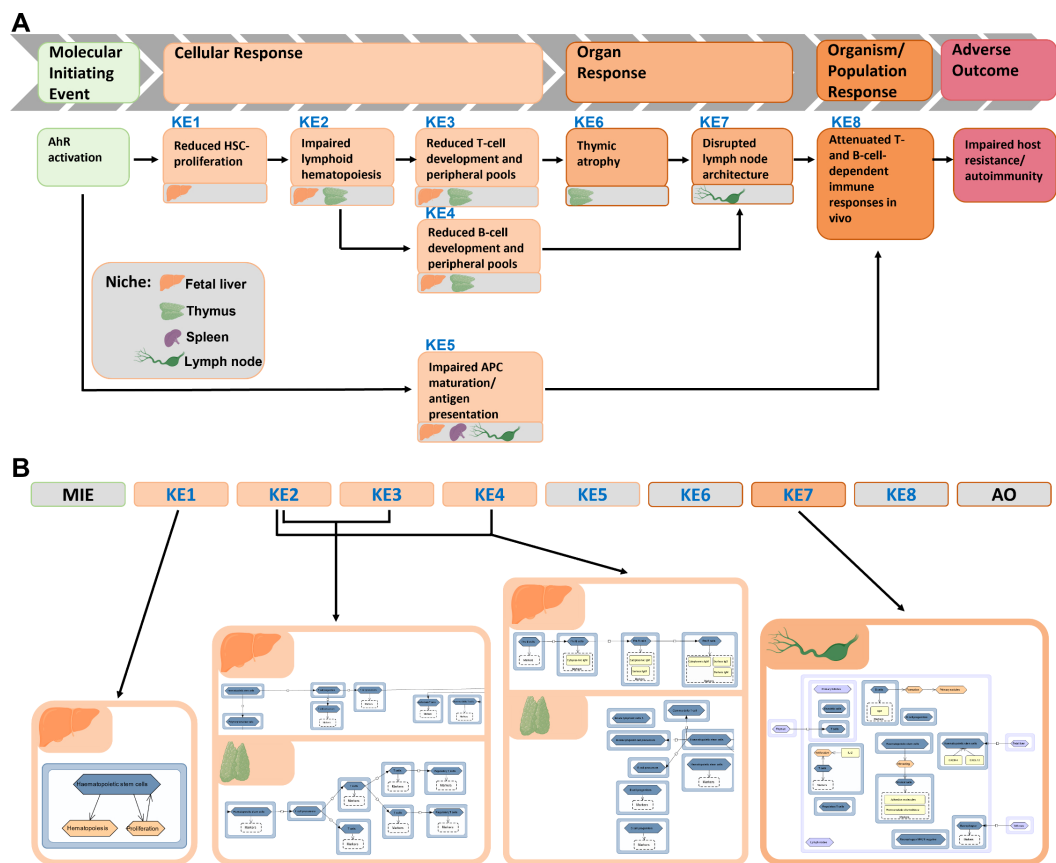


FIGURE 9

Putative AOP linking AhR activation to immunosuppression through disrupted lymphatic development and function. **(A)** Schematic representation of a putative adverse outcome pathway (AOP) describing how activation of the aryl hydrocarbon receptor (AhR) acts as a molecular initiating event (MIE), triggering a cascade of key events (KE1-KE8) that affect immune development. Early events include reduced hematopoietic stem cell proliferation (KE1) and impaired lymphatic hematopoiesis (KE2), followed by reduced T-cell (KE3) and B-cell (KE4) development and impaired maturation and antigen presentation of antigen-presenting cells (APCs, KE5). These cellular alterations manifest at the organ level as thymus atrophy (KE6) and disrupted lymph node architecture (KE7), leading to attenuated T- and B-cell-dependent immune responses that occur *in vivo* (KE8). The adverse outcome (AO) is reduced host resistance or autoimmunity. Relevant anatomical niches (fetal liver, thymus, spleen and lymph nodes) are indicated by symbols. **(B)** Integration of HIDmap elements into the putative AOP as a multi-layered visualization. Individual KEs are represented by specific HIDmap modules at the cellular or organ level, enabling structured mapping of molecular, cellular, and functional processes. To find the examples displayed, enter the following unique element numbers into the search field of the HIDmap: element:81052 (KE1); element:81139 (fetal liver), element:81780 (thymus) (KE2 + 3); element:81202 (fetal liver), element:81852 (thymus) (KE2 + 4); element:81932 (KE7).

3.4 Limitations and future directions

The current version of the HIDmap focuses on prenatal immune system development and is based on literature published between 2017 and 2022. Earlier and more recent studies are currently not represented. Future updates will incorporate newly available datasets through collaborative efforts within the EU-funded Horizon Europe project “Partnership for the Assessment of Risks from Chemicals” (PARC) (100), including the integration of literature related to monogenic immune disorders using resources from the International Union of Immunological Societies (IUIS) (101). Temporal resolution is currently limited by the underlying map architecture. Future updates will include expanded datasets and community-driven refinement of the resource. With respect to AOP development, the HIDmap primarily captures cellular processes and molecular interactions relevant to upstream and cell-based KEs. Higher-level events at the organ or organism level are only represented to a limited extent and therefore require complementary data sources.

4 Conclusion

In summary, the HIDmap represents a structured, human-centered framework for exploring prenatal human immune development and provides a curated digital resource integrating developmental immune processes across biological compartments during prenatal immune development. Specifically, the HIDmap addresses critical knowledge gaps identified by regulatory agencies (e.g., ECHA KARC) by providing structured, expert-curated data on immune ontogeny. Its modular, three-layered architecture enables multi-scale investigation ranging from molecular markers to organ-level processes. In addition, integration into the MINERVA platform ensures accessibility, interactivity, and community-driven sustainability. By embedding mechanistic evidence in a digital environment, the HIDmap makes biological complexity of DIT more accessible, thereby providing new opportunities for the future development of DIT-related AOPs and NAMs and offering a useful framework for mechanistic research in developmental immunotoxicology. While still at the early, exploratory stage, the

HIDmap aligns with ongoing efforts to advance mechanism-based, human-relevant, and animal-free approaches to hazard identification and aligns with the 3R principles and the EU roadmap of phasing out animal experiments.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Author contributions

CS: Data curation, Writing – review & editing, Methodology, Writing – original draft, Visualization, Investigation. LL: Investigation, Methodology, Supervision, Software, Writing – review & editing, Visualization. EK: Writing – review & editing, Methodology. MO: Methodology, Writing – review & editing, Data curation, Investigation, Software, Visualization. ERC: Writing – review & editing. EC: Writing – review & editing, Validation. LG: Writing – review & editing. BS: Writing – review & editing. SF: Writing – review & editing, Funding acquisition. QL: Writing – review & editing, Funding acquisition. FG: Writing – review & editing, Funding acquisition. EF: Conceptualization, Funding acquisition, Writing – review & editing. KK: Validation, Writing – review & editing. JT: Supervision, Conceptualization, Visualization, Methodology, Writing – original draft, Project administration, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The work was funded by the IUF - Leibniz Research Institute for Environmental Medicine. This work also received funding from the European Research Council under the European Union's Horizon 2020 Framework Program (H2020/2014-2021)/ERC grant agreement No 772418 (INSITE).

Acknowledgments

The IUF is funded by the federal and state governments - the Ministry of Culture and Science of North Rhine-Westphalia (MKW) and the Federal Ministry of Research, Technology and Space (BMFTR). The online browsing was supported by the MINERVA team at the Bioinformatics Core Facility of the Luxembourg Centre for Systems Biomedicine, within the framework of ELIXIR-LU framework (<https://elixir-luxembourg.org>). All figures were created using [BioRender.com](https://biorender.com). We acknowledge the support of the International DIT Alternatives Working Group led by the Center of Alternatives (CAAT) to Animal Testing US and the European consortium

Partnership for Assessment of Risk from Chemicals (PARC), a key stakeholder in the continuation of this work. The HIDmap map is freely available via the MINERVA platform (<https://immunedev.elixir340.luxembourg.org/minerva/>). It is licensed under the Creative Commons Attribution 4.0 International 341 (CC BY 4.0) License (<https://creativecommons.org/licenses/by/4.0/>).

Conflict of interest

Authors EF and KK were employed by company DNTOX GmbH. SF, QL, and FG are employees of Clariant and were involved in the review and approval of the final manuscript.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that this work received funding from Clariant Produkte (Deutschland) GmbH. The funder was involved in the review and approval of the final manuscript.

The handling editor VJJ declared a past co-authorship with the author EC.

The authors MO, EC, LG, EF, KK declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2026.1741650/full#supplementary-material>

References

- Balaur I, Welter D, Rougny A, Inau ET, Mazein A, Ghosh S, et al. Fair assessment of disease maps fosters open science and scientific crowdsourcing in systems biomedicine. *Sci Data*. (2025) 12:1–7. doi: 10.1038/s41597-025-05147-w
- Wang X, Li N, Ma M, Han Y, Rao K. Immunotoxicity *in vitro* assays for environmental pollutants under paradigm shift in toxicity tests. *Int J Environ Res Public Health*. (2023) 20. doi: 10.3390/ijerph20010273
- Hossain Z, Reza AHMM, Qasem WA, Friel JK, Omri A. Development of the immune system in the human embryo. *Pediatr Res*. (2022) 92:951–5. doi: 10.1038/s41390-022-01940-0
- Jain N. The early life education of the immune system: Moms, microbes and (missed) opportunities. *Gut Microbes*. (2020) 12:1–20. doi: 10.1080/19490976.2020.1824564
- Feyaerts D, Urbschat C, Gaudillière B, Stelzer IA. Establishment of tissue-resident immune populations in the fetus. *Semin Immunopathol*. (2022) 44:747–66. doi: 10.1007/s00281-022-00931-x
- Zheng H, Chen Y, Luo Q, Zhang J, Huang M, Xu Y, et al. Generating hematopoietic cells from human pluripotent stem cells: approaches, progress and challenges. *Cell Regen*. (2023) 12:1–21. doi: 10.1186/s13619-023-00175-6
- Zhang X, Zhivaki D, Lo-Man R. Unique aspects of the perinatal immune system. *Nat Rev Immunol*. (2017) 17:495–507. doi: 10.1038/nri.2017.54
- Soares-da-Silva F, Peixoto M, Cumano A, Pinto-do-Ó P. Crosstalk between the hepatic and hematopoietic systems during embryonic development. *Front Cell Dev Biol*. (2020) 8. doi: 10.3389/fcell.2020.00612
- Tang MH, Ligthart I, Varga S, Lebeer S, van Overveld FJ, Rijkers GT. Mutual interactions between microbiota and the human immune system during the first 1000 days of life. *Biol (Basel)*. (2025) 14:1–34. doi: 10.3390/biology14030299
- Cain TL, Derecka M, McKinney-Freeman S. The role of the haematopoietic stem cell niche in development and ageing. *Nat Rev Mol Cell Biol*. (2025) 26:32–50. doi: 10.1038/s41580-024-00770-8
- Skaggs H, Chellman GJ, Collinge M, Enright B, Fuller CL, Krayner J, et al. Comparison of immune system development in nonclinical species and humans: Closing information gaps for immunotoxicity testing and human translatability. *Reprod Toxicol*. (2019) 89:178–88. doi: 10.1016/j.reprotox.2019.06.005
- Kuper CF, van Bilsen J, Nossen H, Houben G, Garthoff J, Wolterbeek A. Development of immune organs and functioning in humans and test animals: Implications for immune intervention studies. *Reprod Toxicol*. (2016) 64:180–90. doi: 10.1016/j.reprotox.2016.06.002
- Nguyen H-M, Rogers K, Collinge M. Comparative immune system development. In: McQueen CA, editor. *Comprehensive Toxicology*, 4th ed. Amsterdam: Elsevier Ltd (2024). p. 85–105. doi: 10.1016/B978-0-323-95488-4.00173-X
- Hochstenbach K, Van Leeuwen DM, Gmuender H, Gottschalk RW, Stølevik SB, Nygaard UC, et al. Toxicogenomic profiles in relation to maternal immunotoxic exposure and immune functionality in newborns. *Toxicol Sci*. (2012) 129:315–24. doi: 10.1093/toxsci/kfs214
- Corsini E, Iulini M, Galbiati V, Maddalon A, Pappalardo F, Russo G, et al. Efsa project on the use of NAMs to explore the immunotoxicity of PFAS. *EFSA Support Publ*. (2024) 21. doi: 10.2903/sp.efsa.2024.en-8926
- DeWitt JC, Sarah J, Blossom LAS. Exposure to per- and polyfluoroalkyl substances leads to immunotoxicity: Epidemiological and toxicological evidence. *J Expo Sci Env Epidemiol*. (2019) 29:148–56. doi: 10.1038/s41370-018-0097-y
- Abraham K, Mielke H, Fromme H, Völkel W, Menzel J, Peiser M, et al. Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: Associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. *Arch Toxicol*. (2020) 94:2131–47. doi: 10.1007/s00204-020-02715-4
- Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Budtz-Jørgensen E. Serum vaccine antibody concentrations in adolescents exposed to perfluorinated compounds. *Environ Health Perspect*. (2017) 125:1–7. doi: 10.1289/EHP275
- Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E, et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. *J Immunotoxicol*. (2016) 13:270–3. doi: 10.3109/1547691X.2015.1067259
- European Chemicals Agency (ECHA). Key areas of regulatory challenge. (2023). doi: 10.2823/568850
- Chief editor: Wim De Coen, European Chemicals Agency (ECHA). Key areas of regulatory challenge. (2024). doi: 10.2823/858284
- Chief editor: Wim De Coen, European Chemicals Agency (ECHA). Key areas of regulatory challenge. (2025). doi: 10.2823/8572710
- Iulini M, de Bruijn V, Hurem S, Nygaard UC, Kerdine-Römer S, Spruck C, et al. From animal models to NAMs: a paradigm shift in developmental immunotoxicity testing. *Arch Toxicol*. (2026). doi: 10.1007/s00204-026-04424-w
- Villeneuve DL, Crump D, Hecker M, Hutchinson TH, Lalone CA, Landesmann B, et al. Adverse outcome pathway (AOP) development I : strategies and principles. (2014) 142:312–20. doi: 10.1093/toxsci/kfu199
- Villeneuve DL, Crump D, Hecker M, Hutchinson TH, Lalone CA, Landesmann B, et al. Adverse outcome pathway development II : best practices. (2014) 142:321–30. doi: 10.1093/toxsci/kfu200
- Jaylet T, Coustillet T, Smith NM, Viviani B, Lindeman B, Vergauwen L, et al. Comprehensive mapping of the AOP-Wiki database: identifying biological and disease gaps. (2024) 6:1–22. doi: 10.3389/ftox.2024.1285768
- Pitzer EM, Shafer TJ, Herr DW. Neurotoxicology identification of neurotoxicology (NT)/ developmental neurotoxicology (DNT) adverse outcome pathways and key event linkages with *in vitro* DNT screening assays. *Neurotoxicology*. (2023) 99:184–94. doi: 10.1016/j.neuro.2023.10.007
- Bajard L, Adamovsky O, Audouze K, Baken K, Barouki R, Beltman JB, et al. Application of AOPs to assist regulatory assessment of chemical risks – Case studies, needs and recommendations. (2023) 217:. doi: 10.1016/j.envres.2022.114650
- Hernandez-Jerez A, Adriaanse P, Aldrich A, Berny P, Coja T, Duquesne S, et al. Development of integrated approaches to testing and assessment (IATA) case studies on developmental neurotoxicity (DNT) risk assessment. *EFSA J*. (2021) 19. doi: 10.2903/j.efsa.2021.6599
- Blum J, Masjosthusmann S, Bartmann K, Bendt F, Dolde X, Dönmez A, et al. Establishment of a human cell-based *in vitro* battery to assess developmental neurotoxicity hazard of chemicals. *Chemosphere*. (2023) 311. doi: 10.1016/j.chemosphere.2022.137035
- Masjosthusmann S, Blum J, Bartmann K, Dolde X, Holzer A, Stürzl L, et al. Establishment of an a priori protocol for the implementation and interpretation of an *in-vitro* testing battery for the assessment of developmental neurotoxicity. *EFSA Support Publ*. (2020) 17. doi: 10.2903/sp.efsa.2020.en-1938
- Koch K, Bartmann K, Hartmann J, Kapr J, Klose J, Kuchovská E, et al. Scientific validation of human neurosphere assays for developmental neurotoxicity evaluation. *Front Toxicol*. (2022) 4. doi: 10.3389/ftox.2022.816370
- Lein P, Locke P, Goldberg A. Meeting report: Alternatives for developmental neurotoxicity testing. *Environ Health Perspect*. (2007) 115:764–8. doi: 10.1289/ehp.9841
- Fritsche E, Grandjean P, Crofton KM, Aschner M, Goldberg A, Heinonen T, et al. Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes. *Toxicol Appl Pharmacol*. (2018) 354:3–6. doi: 10.1016/j.taap.2018.02.004
- Bal-Price A, Hogberg HT, Crofton KM, Daneshian M, FitzGerald RE, Fritsche E, et al. Recommendation on test readiness criteria for new approach methods (NAM) in toxicology: exemplified for developmental neurotoxicity (DNT). *ALTEX*. (2018) 35:306–52. doi: 10.14573/altex.1712081
- Staumont B, Ladeira L, Gamba A, Heusinkveld HJ, Piersma A, Fritsche E, et al. Mapping physiology: a systems biology approach for the 1 development of alternative methods in toxicology (2025). Available online at: <https://www.oecd.org/en/topics/sub-issues/testing-of-chemicals/adverse-outcome-pathways.html> (Accessed October 08, 2025).
- Vinken M, Benfenati E, Busquet F, Castell J, Clevert DA, de Kok TM, et al. Safer chemicals using less animals: Kick-off of the European ONTOX project. *Toxicology*. (2021) 458:1–7. doi: 10.1016/j.tox.2021.152846
- Ostaszewski M, Gebel S, Kuperstein I, Mazein A, Zinovyev A, Dogrusoz U, et al. Community-driven roadmap for integrated disease maps. *Brief Bioinform*. (2019) 20:659–70. doi: 10.1093/bib/bby024
- Mazein A, Ostaszewski M, Kuperstein I, Watterson S, Le Novère N, Lefaudeux D, et al. Systems medicine disease maps: Community-driven comprehensive representation of disease mechanisms. *NPJ Syst Biol Appl*. (2018) 4. doi: 10.1038/s41540-018-0059-y
- Mazein A, Shoaib M, Alb M, Sakellariou C, Sommer C, Sewald K, et al. Using interactive platforms to encode, manage and explore immune-related adverse outcome pathways. *J Immunotoxicol*. (2024) 21:S5–S12. doi: 10.1080/1547691X.2024.2345154
- Basha S, Surendran N, Pichichero M. Immune responses in neonates. *Expert Rev Clin Immunol*. (2014) 10:1171–84. doi: 10.1586/1744666X.2014.942288
- Calvanese V, Capellera-Garcia S, Ma F, Fares I, Liebscher S, Ng ES, et al. Mapping human haematopoietic stem cells from haemogenic endothelium to birth. *Nature*. (2022) 604:534–40. doi: 10.1038/s41586-022-04571-x
- Copley MR, Eaves CJ. Developmental changes in hematopoietic stem cell properties. *Exp Mol Med*. (2013) 45:1–10. doi: 10.1038/emm.2013.98
- De Moraes-Pinto MI, Suano-Souza F, Aranda CS. Immune system: Development and acquisition of immunological competence. *J Pediatr (Rio J)*. (2021) 97:S59–66. doi: 10.1016/j.jped.2020.10.006
- Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy Eur J Allergy Clin Immunol*. (2000) 55:688–97. doi: 10.1034/j.1398-9995.2000.00118.x

46. Popescu DM, Botting RA, Stephenson E, Green K, Webb S, Jardine L, et al. Decoding human fetal liver haematopoiesis. *Nature*. (2019) 574:365–71. doi: 10.1038/s41586-019-1652-y
47. Suo C, Dann E, Goh I, Jardine L, Kleshchevnikov V, Park JE, et al. Mapping the developing human immune system across organs. *Science* (80-). (2022) 376. doi: 10.1126/science.abo0510
48. Ygberg S, Nilsson A. The developing immune system - From foetus to toddler. *Acta Paediatr Int J Paediatr*. (2012) 101:120–7. doi: 10.1111/j.1651-2227.2011.02494.x
49. Bozada T, Borden J, Workman J, Del Cid M, Malinowski J, Luechtefeld T. Sysrev: A FAIR platform for data curation and systematic evidence review. *Front Artif Intell*. (2021) 4. doi: 10.3389/frai.2021.685298
50. Bian Z, Gong Y, Huang T, Lee CZW, Bian L, Bai Z, et al. Deciphering human macrophage development at single-cell resolution. *Nature*. (2020) 582:571–6. doi: 10.1038/s41586-020-2316-7
51. Dege C, Fegan KH, Creamer JP, Berrien-Elliott MM, Luff SA, Kim D, et al. Potently cytotoxic natural killer cells initially emerge from erythro-myeloid progenitors during mammalian development. (2021) 53:229–39. doi: 10.1016/j.devcel.2020.02.016
52. Kang HJ, Mesquitta WT, Jung HS, Moskvina OV, Thomson JA, Slukvin II. GATA2 is dispensable for specification of hemogenic endothelium but promotes endothelial-to-hematopoietic transition. *Stem Cell Rep*. (2018) 11:197–211. doi: 10.1016/j.stemcr.2018.05.002
53. Uenishi GI, Jung HS, Slukvin II. Assessment of endothelial-to-hematopoietic transition of individual hemogenic endothelium and bulk populations in defined conditions. In: Kannan N, Beer PA, editors. *Stem Cell Assays. Methods in Molecular Biology*. (2022) Vol. 2454. New York, NY: Humana; 2022. p. 103–12. doi: 10.1007/978-1-0716-1979-7_8
54. Jardine L, Hani M. Reconstructing human DC, monocyte and macrophage development in utero using single cell technologies. *Mol Immun*. (2020) 123:1–6. doi: 10.1016/j.molimm.2020.04.023
55. Kumar BV, Connors T, Farber DL. Human T cell development, localization, and function throughout life. *Immunity*. (2019) 48:202–13. doi: 10.1016/j.immuni.2018.01.007
56. Lewis K, Yoshimoto M, Takebe T. Fetal liver hematopoiesis: from development to delivery. *Stem Cell Res Ther*. (2021) 12:139. doi: 10.1186/s13287-021-02189-w
57. Najafi S, Ghanavat M, Saki N. The effect of inflammatory factors and their inhibitors on the hematopoietic stem cells fate. *Cell Biol Int*. (2021) 45(5):900–12. doi: 10.1002/cbin.11545
58. Park J, Jardine L, Gottgens B. Prenatal development of human immunity. *Science*. (2022) 368:600–3. doi: 10.1126/science.aaz9330
59. Rothenberg EV. Single-cell insights into the hematopoietic generation of T lymphocyte precursors in mouse and man. *Exp Hematol*. (2022) 95:1–12. doi: 10.1016/j.exphem.2020.12.005
60. Xin Z, Zhang W, Gong S, Zhu J, Li Y. Mapping human pluripotent stem cell-derived erythroid differentiation by single-cell transcriptome analysis. *Genomics Proteomics Bioinf*. (2021) 19:358–76. doi: 10.1016/j.gpb.2021.03.009
61. Zeng Y, Liu C, Gong Y, Lan Y, Liu B, Zeng Y, et al. Single-cell RNA sequencing resolves spatiotemporal development of pre-thymic lymphoid progenitors and thymus organogenesis in human embryos resource single-cell RNA sequencing resolves spatiotemporal development of pre-thymic lymphoid progenitors and thym. *Immunity*. (2019) 51:930–948.e6. doi: 10.1016/j.immuni.2019.09.008
62. Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, et al. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics*. (2003) 19:524–31. doi: 10.1093/bioinformatics/btg015
63. Hoksza D, Gawron P, Ostaszewski M, Hasenauer J, Schneider R. Closing the gap between formats for storing layout information in systems biology. *Brief Bioinform*. (2019) 21:1249–60. doi: 10.1093/bib/bbz067
64. Junker A, Sorokin A, Czauderna T, Schreiber F, Mazein A. Wiring diagrams in biology: towards the standardized representation of biological information. *Trends Biotechnol*. (2012) 30:555–7. doi: 10.1016/j.tibtech.2012.08.003
65. Novère NL, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, et al. The systems biology graphical notation. *Nat Biotechnol*. (2009) 27:735–41. doi: 10.1038/nbt.1558
66. Touré V, Le Novère N, Waltemath D, Wolkenhauer O. Quick tips for creating effective and impactful biological pathways using the systems biology graphical notation. *PLoS Comput Biol*. (2018) 14:1–6. doi: 10.1371/journal.pcbi.1005740
67. Juty N, Le Novère N, Laibe C. Identifiers.org and MIRIAM Registry: community resources to provide persistent identification. *Nucleic Acids Res*. (2012) 40:580–6. doi: 10.1093/nar/gkr1097
68. Gawron P, Ostaszewski M, Satagopam V, Gebel S, Mazein A, Kuzma M, et al. MINERVA—a platform for visualization and curation of molecular interaction networks. *NPJ Syst Biol Appl*. (2016) 2:1–6. doi: 10.1038/npjbsa.2016.20
69. Acencio ML, Lopata O, Hemedan AA, Dand N, Weidinger S, Paternoster L, et al. The inflammatory skin disease map: an interactive computational resource focused on molecular mechanisms in psoriasis and atopic dermatitis. *Br J Dermatol*. (2025) 194:597–9. doi: 10.1093/bjd/ljaf491
70. Seal RL, Braschi B, Gray K, Jones TEM, Tweedie S, Haim-Vilmovsky L, et al. Genenames.org: the HGNC resources in 2023. *Nucleic Acids Res*. (2023) 51:D1003–9. doi: 10.1093/nar/gkac888
71. OECD. Guidance document on developing and assessing adverse outcome pathways. (2017).
72. Wilkinson MD, Dumontier M, Aalbersberg I, Appleton G, Axton M, Baak A, et al. Comment: the FAIR guiding principles for scientific data management and stewardship. *Sci Data*. (2016) 3:1–9. doi: 10.1038/sdata.2016.18
73. Niarakis A, Ostaszewski M, Mazein A, Kuperstein I, Kutnom M, Gillespie ME, et al. Drug-target identification in COVID-19 disease mechanisms using computational systems biology approaches. *Front Immunol*. (2023) 14. doi: 10.3389/fimmu.2023.1282859
74. Fujita KA, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, et al. Integrating pathways of Parkinson's disease in a molecular interaction map. *Mol Neurobiol*. (2014) 49:88–102. doi: 10.1007/s12035-013-8489-4
75. Mazein A, Lopata O, Reiche K, Sewald K, Alb M, Sakellariou C, et al. An explorable model of an adverse outcome pathway of cytokine release syndrome related to the administration of immunomodulatory biotherapeutics and cellular therapies. *Front Immunol*. (2025) 16. doi: 10.3389/fimmu.2025.1601670
76. AOP-Wiki. Thyroperoxidase Inhibition Leading to Decreased Amphibian Metamorphosis (AOP 154). Available online at: <https://aopwiki.org/aops/154> (Accessed October 15, 2025).
77. AOP-Wiki. Impaired IL-1R1 Signaling Leading to Impaired T-Cell Dependent Antibody Response (AOP 277). Available online at: <https://aopwiki.org/aops/277> (Accessed October 15, 2025).
78. Marshall NB, Vorachek WR, Stepan LB, Mourich DV, Kerkvliet NI. Functional characterization and gene expression analysis of CD4+ CD25+ regulatory T cells generated in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Immunol*. (2008) 181:1–7. doi: 10.4049/jimmunol.181.4.2382
79. Lindsey S, Papoutsakis ET. The evolving role of the aryl hydrocarbon receptor (AHR) in the normophysiology of hematopoiesis. *Stem Cell Rev Rep*. (2012) 8:1223–35. doi: 10.1007/s12015-012-9384-5
80. Khan DMIO, Crawford RB, Kaminski NE. Regulation of *in vitro* human hematopoietic differentiation by dioxin-like compounds. *Toxicology*. (2025) 514:154136. doi: 10.1016/j.tox.2025.154136
81. Esser C, Temchura V, Majora M, Hunderiker C, Schwärzler C, Günther U. Signaling via the AHR leads to enhanced usage of CD44v10 by murine fetal thymic emigrants: possible role for CD44 in emigration. *Int Immunopharmacol*. (2004) 4:805–18. doi: 10.1016/j.intimp.2004.03.006
82. Khan DMIO, Karmaus PWF, Bach A, Crawford RB, Kaminski NE. An *in vitro* model of human hematopoiesis identifies a regulatory role for the aryl hydrocarbon receptor. *Blood Adv*. (2023) 7:6253–65. doi: 10.1182/bloodadvances.2023010169
83. Casado FL, Singh KP, Gasiewicz TA. Aryl hydrocarbon receptor activation in hematopoietic stem/progenitor cells alters cell function and pathway-specific gene modulation reflecting changes in cellular trafficking and migration. *Mol Pharmacol*. (2011) 80:673–82. doi: 10.1124/mol.111.071381
84. Silbergeld EK, Mattison DR. Experimental and clinical studies on the reproductive toxicology of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Am J Ind Med*. (1987) 11:131–44. doi: 10.1002/ajim.4700110203
85. Holladay SD, Lindstrom P, Blaylock BL, Comment CE, Germolec DR, Heindell JJ, et al. Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-p-dioxin (TCDD). *Teratology*. (1991) 44:385–93. doi: 10.1002/tera.1420440405
86. Fiorito F, Santamaria R, Irace C, De Martino L, Iovane G. 2,3,7,8-Tetrachlorodibenzo-P-dioxin and the viral infection. *Environ Res*. (2017) 153:27–34. doi: 10.1016/j.envres.2016.11.004
87. Zhou J, Blevins LK, Crawford RB, Kaminski NE. Role of programmed cell death protein-1 and lymphocyte specific protein tyrosine kinase in the aryl hydrocarbon receptor-mediated impairment of the IgM response in human CD5+ innate-like B cells. *Front Immunol*. (2022) 13. doi: 10.3389/fimmu.2022.884203
88. Lumms ZL, Henningsen G. Modulation of T-cell ontogeny by transplacental benzo(a)pyrene. *Int J Immunopharmacol*. (1995) 17:339–50. doi: 10.1016/0192-0561(94)00098-9
89. Mustafa A, Holladay SD, Goff M, Witonsky SG, Kerr R, Reilly CM, et al. An enhanced postnatal autoimmune profile in 24 week-old C57BL/6 mice developmentally exposed to TCDD. *Toxicol Appl Pharmacol*. (2008) 23:1–7. doi: 10.1016/j.taap.2008.04.015
90. Rodriguez JW, Kiriln WG, Wirsy YG, Matheravidathu S, Hodge TW, Urso P. Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. *Immunopharmacol Immunotoxicol*. (1999) 21:379–96. doi: 10.3109/08923979909052769
91. Gehrs BC, Riddle MM, Williams WC, Smialowicz RJ. Erratum: alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin II. Effects on the pup and the adult. *Toxicology*. (1998) 130:71. doi: 10.1016/S0300-483X(98)00113-9

92. Grasman KA, Whitacre LL. Effects of PCB 126 on thymocyte surface marker expression and immune organ development in chicken embryos. *J Toxicol Environ Heal - Part A*. (2001) 62:191–206. doi: 10.1080/009841001458307
93. Van Grevenynghe J, Bernard M, Langouet S, Le Berre C, Fest T, Fardel O. Human CD34-positive hematopoietic stem cells constitute targets for carcinogenic polycyclic aromatic hydrocarbons. *J Pharmacol Exp Ther*. (2005) 314:693–702. doi: 10.1124/jpet.105.084780
94. Wang C, Petriello MC, Zhu B, Hennig B. PCB 126 induces monocyte/macrophage polarization and inflammation through AhR and NF- κ B pathways. *Toxicol Appl Pharmacol*. (2019) 367:71–81. doi: 10.1016/j.taap.2019.02.006
95. Kanehisa M, Furumichi M, Sato Y, Matsuura Y, Ishiguro-Watanabe M. KEGG: biological systems database as a model of the real world. *Nucleic Acids Res*. (2025) 53:D672–7. doi: 10.1093/nar/gkae909
96. Ragueneau E, Gong C, Sinquin P, Sevilla C, Beavers D, Grentner A, et al. The reactome knowledgebase 2026. *Nucleic Acids Res*. (2026) 54:D673–81. doi: 10.1093/nar/gkaf1223
97. Agrawa A, Balci H, Hanspers KCS, Martens M, Slenter DN, Digles D, et al. WikiPathways 2024: next generation pathway database. *Nucleic Acids Res*. (2024) 52:679–89. doi: 10.1093/nar/gkad960
98. Krämer A, Green J, Pollard J, Tugendreich S. Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics*. (2014) 30:523–30. doi: 10.1093/bioinformatics/btt703
99. Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, et al. The human cell atlas. *Elife*. (2017), 1–30. doi: 10.7554/eLife.27041.001
100. Smith NM, Bruijn VMP, Vandebriel RJ, Hargitai R, Lumniczky K, Janssen AWF, et al. New approach methodologies (NAMs) to support regulatory assessment of developmental immunotoxicity – a new PARC project. (2026) 8:1–11. doi: 10.3389/ftox.2026.1740390
101. Poli MC, Aksentijevich I, Bousfiha AA, Cunningham-Rundles C, Hambleton S, Klein C, et al. Human inborn errors of immunity: 2022 update on the classification from the International Union of Immunological Societies Expert Committee. *J Clin Immunol*. (2022) 42:1473–507. doi: 10.1007/s10875-022-01289-3

Glossary

3R	Replacement, reduction and refinement	KE	Key event
AGM	Aorta-gonad-mesonephros	KEGG	Kyoto Encyclopedia of Genes and Genomes
AhR	Aryl hydrocarbon receptor	MHC	Major histocompatibility complex
AOP	Adverse outcome pathway	MIE	Molecular initiating event
BaP	Benzo[a]pyrene	MINERVA	Molecular interaction network visualization
CRS	Cytokine release syndrome	MIRIAM	Minimal information requested in the annotation of biochemical models
DIT	Developmental immunotoxicology	MPP	Multipotent progenitors
DNT	Developmental neurotoxicity	NAM	New approach methodology
ECHA	European Chemicals Agency	NIPH	Norwegian Institute of Public Health
EFSA	European Food and Safety Authority	ONTOX	Ontology-driven and artificial intelligence-based repeated dose toxicity testing of chemicals for next generation risk assessment
EMP	erythro-myeloid progenitors		
FAIR	Findable, accessible, interoperable, and reusable	PARC	Partnership for the Assessment of Risks from Chemicals
HCA	Human Cell Atlas	PCB	Polychlorinated biphenyls
HGNC	HUGO gene nomenclature committee	PFAS	Per- and polyfluorinated alkyl substances
HIDmap	Human immune system development map	PM	Physiological map
HSC	Hematopoietic stem cell	PMID	PubMed identifier
HUGO	Human Genome Organization	SBGN	Systems biology graphical notation
IGI	Inborn Errors of Immunity Committee	SBML	Systems biology markup language
IPA	Ingenuity Pathway Analysis	TCDD	2,3,7,8-Tetrachlorodibenzodioxin.
IUIS	International Union of Immunological Societies		
KARC	Key Areas of Regulatory Challenge		