Macrophages as targets of developmental immunotoxicity

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Abstract
Introduction
Developmental immunotoxicity and foetal programming of the immune system are recognised as significant factors affecting the risk of both childhood and adult diseases. In particular, the elevated risk of non-communicable diseases such as atherosclerosis, lupus, psoriasis, rheumatoid arthritis, neurodegenerative diseases, celiac disease, inflammatory bowel disease and asthma is a concern. Misregulated inflammation is often associated with these conditions. Immunotoxicity, in general, has historically emphasised assessment of lymphoid structure and function over innate immune dysfunction. However, given (1) the role of inflammation in developmental immunotoxicity-associated disease and (2) the presence of specialised resident macrophage populations in most target tissues of non-communicable diseases, the tissue of macrophage targeting via developmental immunotoxicity is of interest. This mini-review is among the first to focus solely on macrophages and developmental immunotoxicity.

Discussion
Developmental immunotoxicity studies were examined for inclusion of innate immune and/or macrophage assessment. Taken together, the studies reveal a wealth of information on macrophage-targeted developmental immunotoxicity and suggest that macrophage populations are particularly sensitive for early life environmental insult and foetal programming for dysfunction. Numerous perinatal environmental factors (diet, pollutants, drugs, physical and psychological stressors, and maternal infections) target macrophages producing serious later-life adverse outcomes. Additionally, dysfunction can involve specialised macrophage populations such as microglia, Langerhans cells, alveolar macrophages, Kupffer cells and reproductive organ macrophages.

Conclusion
The findings suggest that developmental immunotoxicity targeting of macrophages should be a first-line consideration in health risk assessment rather than a second-tier measure of developmental immunotoxicity.

Introduction
Research on disruption of immune development leading to adverse later-life outcomes dates back to at least the 1970s and has fallen under the rubric of developmental immunotoxicity (DIT). Agents of immune disruption include environmental chemicals, drugs, diet, physical factors (e.g. ultraviolet (UV) radiation) and psychological factors. DIT is an important component in the broader landscape of what is known as the developmental origins of adult health and disease. This idea of early life programming of myriad later-life diseases evolved from the more narrowly cast ‘Barker hypothesis’, which focused on early life nutritional risk factors of cardiovascular disease outcomes1. In the case of DIT, early insult can take two somewhat different forms. Real-time disruption of critical maturational events can occur such as interference with positive and negative thymic selection in the thymus. This would be likely to increase the risk of later autoimmune reactions. Additionally, early environmental exposures can produce epigenetic alterations that result in later-life immune dysfunctional phenotypes. These two routes to DIT may well co-exist following certain environmental exposures.

While there are a myriad of potential outcomes resulting from DIT, the most prevalent ones, based on the prevalence of immune dysfunction-related human disease, concern risk of non-communicable diseases (NCDs). Among these are autoimmune, allergic and inflammatory conditions involving virtually every tissue and organ of the body. Examples of some childhood-onset conditions that have been connected to DIT include asthma, allergic rhinitis, food allergies, celiac disease and type 1 diabetes2. Even the later-life onset atherosclerosis appears to have early life roots given that biomarkers of atherosclerosis are evident during childhood3. Misregulated, unresolving inflammation has been suggested as a major factor in risk of immune dysfunction-driven NCDs.

Lympho-centric focus of most developmental immunotoxicity studies and assessments
While a significant number of studies and evaluation have examined DIT, considerably more focus has been placed on adaptive immune responses and lymphoid cell surface markers as indicators of DIT than with innate immune dysfunction. In fact, the most widely used functional assessment for immunotoxicity screening

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in general including DIT has been the T-dependent antibody assays (TDAR) often measuring IgM response to sheep erythrocytes or keyhole limpet haemocyanin as the endpoint\(^4,5\). The argument that has been made is antigen presenting cell (APC) activity is included in the TDAR measure, hence, professional APCs such as macrophages do have some input. But in reality, the key measurements pertaining to risk of immune dysfunction producing NCDs are more likely to fall among macrophages and other innate immune cells responding to challenges in tissues. Given the concern over misregulated inflammation associated with NCDs and potential targeting of tissue macrophages (e.g. microglia, alveolar macrophages and Kupffer cells) by environmental agents, the present mini-review focuses solely on the macrophage as a target of DIT.

There are many biomarkers and assessment tools available for the evaluation of macrophage cell populations and function. A recent review discussed the assays that can be applied to an examination of immunotoxicity involving macrophages\(^6\).

**Discussion**

The author has referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

**Macrophages**

**Distribution and diversity**

Macrophages are among the most widely distributed and diverse cell type of the immune system. Recognition of their many functions has evolved from an original view as a primary vehicle for removing dead and dying cells to a far more global role as a homeostatic regulator of tissues. In fact, because macrophages can take on such a diverse range of form and function and have been given other names connected with their resident tissues during much earlier immunology and cell biology research, their cell-lineage origins had been unclear. Figure 1 illustrates many varied forms of macrophages as distributed in tissues. For the standpoint of toxicant-induced alteration of function, each of these specialised forms may have its own characteristics and sensitivities related to environmental insult. For example, mineral fibres are thought to have a higher persistence rate in the peritoneal cavity versus the lung based on differences in peritoneal versus alveolar macrophage phagocytic activity and subsequent responses\(^7\).

Macrophages serve as an anchor of innate immune function via molecular pattern recognition, phagocytic, chemokine recruitment of cells, cytokine orchestration, metabolic control of the local environment, hormonal modification of surrounding cells, and tumour and virus-infected killing functions. In addition to participating in antigen presentation for adaptive immunity, macrophages also provide an important homeostatic function in virtually all tissues and organs. This includes not only the removal of dead cells and repair processes within tissues but also the sampling at portals of the exposure to the external environment (e.g. airways, gastrointestinal tract and urogenital tract). Macrophages sense microbes allowing them to interact with both the normal commensal microbiota and invading pathogens. They are also a front line in detection as well as in the handling and/or transport of potentially harmful environmental contaminants (e.g. airborne particulate matter). Given their locations in organs and tissues, range of critical

**Figure 1:** Macrophages populate most tissues and organs of the body performing both highly specialised functions and regulating organ homeostasis. Examples of different macrophage populations associated with different tissues are illustrated. Each population may have its own sensitivity and profile for environmentally induced for developmental immunotoxicity.
host-wellness functions and interactions with the environment, it is not surprising that their status can often dictate organ and tissue status.

Their specific distribution and presence in so many tissues means that they are invariably among the cells that first receive significant environmental exposures. Whether it is inhaled diesel exhaust particles or mycobacteria, UVB skin radiation or oral exposure to plasticisers, resident macrophages are at the portal of environmental exposure and are among the first cells to handle the chemical or drug. In fact, the manner in which they first interact with and handle the environmental risk factors can often set the template for the longer term health risks for the host.

Plasticity and polarisation

In responding to challenges to the host, macrophages can shift among a variety of phenotypes to participate in different overlapping phases of first inflammation followed by cell proliferation, angiogenesis and then matrix remodelling. Giorgio argued that macrophages are potentially the cell type with the most phenotypic flexibility and that the balance of shifting among macrophage phenotypes (such as M1 vs. M2 macrophages) is a response to environmental signals. Figure 2 illustrates examples of environmental factors that can promote either M1 or M2 polarisation. Macrophage populations can polarise into largely pro-inflammatory cells (M1) capable of carrying out not only antimicrobial functions but also tissue destruction or, alternatively, the polarisation can skew towards growth promoting cell populations (M2) involved in repair, cell proliferation and healing. M2 macrophages have also been involved with tumour cell proliferation. Subgroups of M2 macrophages have also been defined based on induction signals, cell surface markers and functional distinctions. The extent to which environmental factors can alter the capacity for or balance of macrophage polarisation in response to host challenge can greatly affect the risk of specific pathological outcomes.

Dysfunction among macrophages can vary widely resulting in such adverse outcomes as: (1) misdirected and/or misregulated inflammation causing tissue damage, pathology and loss of function, (2) failure to heal or (3) fibrosis. Ineffective antigen presentation is also a potential functional problem among macrophages.

Reported environmental risk factors for developmental immunotoxicity of macrophages

While DIT studies date back to the 1970s, only subset of these studies included macrophages and/or macrophage-driven functions in the adverse outcome assessment. Despite this limitation, a broad range of environmental risk factors for the DIT of macrophages has been reported in the literature. These are shown in Table 1 including the macrophage populations affected as well as the exposure windows, species and genders examined. It is apparent that the same categories of risk factors producing DIT also target macrophages. Few studies have compared different macrophage populations for differential sensitivity. Since most studies are connected to a specific disease model, analyses are often limited to one specialised population of macrophages located in the target tissue of interest (e.g. alveolar macrophages, testicular macrophages, microglia and Kupffer cells).

Environmental risk factors include metals (lead, cadmium, arsenic, manganese), endocrine disrupting chemicals (e.g. bisphenol A, di-2-ethylhexyl phthalate (DEHP), nonylphenol and 2,3,7,8-tetrachlorodibenzo-p-dioxin), pesticides (e.g. paraquat, chlorpyrifos), endocrine disrupters (e.g. diethylstilbestrol, bisphenol A) and hormones (e.g. oestrogen-endocrine status). The latter suggests that many endocrine disrupting chemicals may similarly affect this pathway.

Figure 2: The polarisation of macrophages between M1 versus M2 cells exerting a significant influence over the host defence and repair activities partitioning antimicrobial, pro-inflammatory activities in tissues versus anti-inflammatory, repair and growth promotion functions that can include even the growth promotion of tumours. Several different classes of environmental factors have been identified that affect this polarisation. These include: drugs, metals and oestrogen-endocrine status. The latter suggests that many endocrine disrupting chemicals may similarly affect this pathway.

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### Table 1  Developmental immunotoxicity of macrophage populations

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<th>Macrophage population</th>
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DE, diesel exhaust; DEHP, di-2-ethylhexyl phthalate; NF-κB, nuclear factor-kappa-B; TLR, toll-like receptor; TNF-α, tumour necrosis factor alpha.
pesticides (chlordane), air pollutants, dietary factors, maternal stressors, component of infectious agents (LPS), alcohol, nicotine, combustion products, volatile organic compounds (e.g. toluene), physical factors (e.g. UVB radiation) and drugs. Some combinations of exposure were also found to produce macrophage toxicity (e.g. diesel exhaust exposure combined with maternal stress or juvenile high fat diet).

As expected, reported alterations of DIT in macrophages cover a wide range of changes including changes in cell number, reduced phagocytic activity, altered toll-like receptor (TLR) expression and/or signalling, skewed cytokine production (often favouring pro-inflammatory cytokine production), altered metabolite production and altered cytotoxic activity towards tumour cells or microbes.

Health ramifications of disrupted macrophage homeostasis: focus on non-communicable diseases

Macrophages appear to play a pivotal role in the promotion of NCDs that compromise a wide range of tissues. Therefore, disruption of normal macrophage seeding of tissues, maturation and acquisition of functional capacities related to tissue homeostasis increase the likelihood of later tissue-focused pathology and disease. Examples of the involvement of dysfunctional macrophage populations with NCDs include: atherosclerosis, cardiovascular diseases, type 2 diabetes, non-alcoholic hepatic steatosis, asthma, chronic obstructive pulmonary disease, obesity and Alzheimer’s disease.

Critical windows of macrophage vulnerability

As with other components of the developing immune system, macrophages in the non-adult appear to be more sensitive to environmental insult and functional alteration than those of the adult. This increased sensitivity of the young (vs. adults) is not restricted only to prenatal development but also includes postnatal (e.g. juvenile) periods of development. For at least one toxicant, macrophages appear to have a broader window of increased sensitivity compared with certain lymphoid populations. Bunn et al. found that prenatal lead exposure in rats across early and late windows of gestation produced persistent macrophage toxicity, whereas, lead-induced T-helper cell alterations were restricted to exposures during later embryonic development. However, there is a dearth of information on distinctions among narrower developmental windows within prenatal, adolescent or juvenile periods. Figure 3 illustrates windows of macrophage sensitivity described for various environmental risk factors from a selection of studies in Table 1. The figure reinforces the importance of both prenatal and postnatal developmental windows during which specific macrophage populations may possess enhanced sensitivity for DIT.

Conclusion

Macrophages are a ready target of DIT and the ramifications of adverse macrophage outcomes of DIT usually result in an increased risk of NCDs. These diseases manifest in a wide array of tissues mostly supported by misregulated inflammation. While macrophages have not been a pivotal focus within immunotoxicology or its sub-category, DIT, there is ample evidence to suggest they deserve more attention. In the handful of studies when comparative sensitivity in early development has been measured across immune system and other cellular components, macrophage parameters are among the most sensitive indicators of toxicity. An example of this is found in Tonk et al., where innate immune inflammatory markers, including those from macrophages, were among the most sensitive indicators of juvenile immunotoxicity to DEHP exhibiting far greater sensitivity than several reproductive endpoints. In the effort to reduce the prevalence of Alzheimer’s disease, a number of studies have focused on the development of interventions that target DIT. A list of potential candidates includes the use of agents that modulate the expression of TLRs, the use of agents that enhance the activity of macrophages, and the use of agents that target the inflammatory response.

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of environmentally mediated NCDs beginning in early life, more attention needs to be paid to the impact of environmental factors on different macrophage populations.

Abbreviations list
APC, antigen presenting cell; DEHP, di-2-ethylhexyl phthalate; DIT, developmental immunotoxicity; NCD, non-communicable diseases; TDAR, T-dependent antibody assays; UV, ultraviolet.

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References