Original Research Article

ENVIRONMENTAL EPIDEMIOLOGY

The EXIMIOUS project—Mapping exposure-induced immune effects: connecting the exposome and the immunome

Steven Ronsmans[a], Karin Sørig Hougaard[b], Tim S. Nawrot[a,c], Michelle Plusquin[c], François Huaux[d], María Jesús Cruz[e], Horatiu Moldovan[f], Steven Verpaele[g], Murali Jayapala[h], Michael Tunney[i], Stéphanie Humblet-Baron[j], Hubert Dirven[k], Unni Cecilie Nygaard[k], Birgitte Lindeman[k], Nur Duale[k], Adrian Liston[l], Esben Meulengracht Flachs[m], Kenneth Kastaniegaard[n], Matthias Ketzel[o], Julia Goetz[p], Jeroen Vanoirbeek[a], Manosij Ghosh[a,*], Peter H. M. Hoet[a,*], The EXIMIOUS Consortium§

Introduction

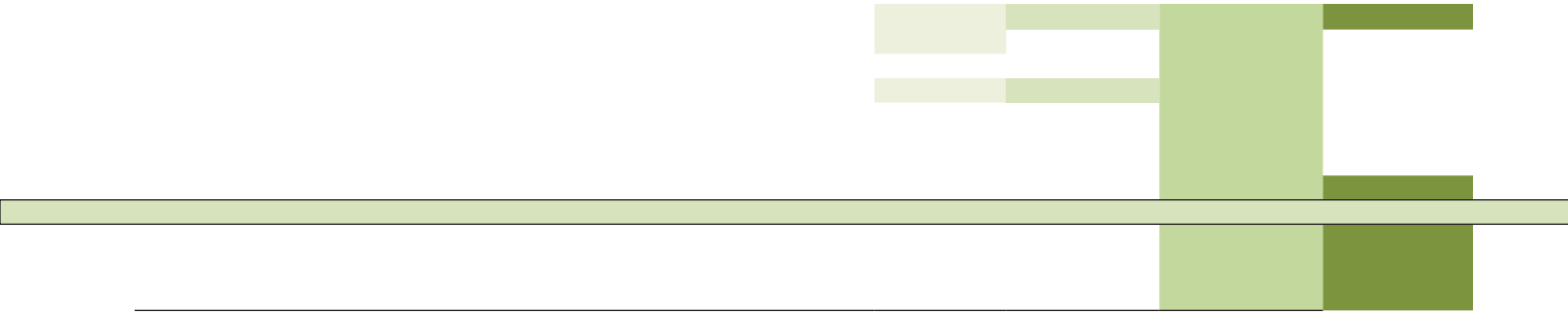
The immune system is vulnerable to adverse effects from environmental exposures. Due to the complexity of the immune system, alterations in its structure or functioning can lead to a range of effects such as immunosuppression—resulting in reduced resistance to infections or tumors—or exaggerated immune responses—which may facilitate hypersensitivity or autoimmunity.[1] The prevalence and societal costs of immune-mediated disorders are rising in the EU.[2,3] Both intrinsic factors—such as genetics, hormones, and age—as well as the environment contribute to the induction, development, and progression of these diseases.[4] However, recent studies, using a systems-immunology approach, have shown that variations in the human immune system are largely driven by nonheritable in uences.[5]

Autoimmune diseases can be considered a model for dissecting the interaction between the immune system and the environment in disease. In autoimmune diseases, there is a speci c, self-reactive immune response—in the form of autoantibodies and/or T-cell responses—that can produce a variety of clinical conditions, depending on the target of the attack. Hundreds of these diseases or syndromes have been described, many of which are organ-speci c, such as autoimmune thyroid disease and type-1 diabetes, while others are systemic—e.g., systemic sclerosis, rheumatoid arthritis, and systemic lupus erythematosus. For many autoimmune diseases the environmental contribution exceeds 50%—and is as high as 95% for some.[6,7] Several reviews have con rmed this link between speci c (single) compounds and immune-related diseases.[8,9] For example, crystalline silica has been linked to a range of autoimmune diseases, such as systemic sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and ANCA-positive vasculitis,[10,11] but also to other immune-mediated diseases such as sarcoidosis.[12] A number of other agents—such as solvents, vinyl chloride, mercury, dioxins, pesticides, plastic-related components, and air pollution—have been associated with autoimmunity.[6,13–15]

[e]Vall d'Hebron Research Institute, Barcelona,

Another group of immune-mediated conditions that have been associated with occupational and environmental exposures are granulomatous diseases, characterized by the development of the focal aggregation of immune cells. In hypersensitivity pneumonitis (HP), granulomas are formed in the lung; in sarcoidosis, they can form in any organ. There is considerable evidence to support the idea that HP and sarcoidosis arise, in genetically susceptible hosts, due to exposure to one or several antigen(s); in HP mainly small organic particles, such as fungi and avian proteins, in sarcoidosis presumably (a combination of) organic and mineral dust particles.[16]

In recent years, the concept of the *exposome* has gained traction, referring to the totality of environmental and occupational exposures from conception onwards.[17,18] Although the concept is theoretically sound, our ability to measure past exposures is limited. Moreover, measuring each agent to which a person has been exposed throughout the entire life course is not feasible at present nor in the near future. Nevertheless, the exposome concept offers an interesting framework. In the EXIMIOUS project (https://www.eximious-h2020.eu/), we strive to assess multiple and combined exposures in a range of different study populations (https://www.eximious-h2020.eu/cohorts/) including general population and birth cohorts (four, Table 1), populations with occupational exposure (three, Table 1) and disease populations v pf9e1lc(j 0.encept of [(cept off f f [(limiFu7.1 rmed.21 Tw [),)wimaidiffer group ofposome con)]TJ 0 Tw 1 FigntaTa12vide5 05j



DOC*X Generation includes the children of all DOC*X members with their birth and health outcomes (>1.2 million children), with follow-up of up to 38 years of age. In the EXIMIOUS project, DOC*X and Generation will be updated until 2018. For this cohort, we will model air-pollution exposure at the individual level, based on residential street addresses.

Occupational populations

Waste worker cohort: The workers in this Danish cohort are exposed to a high diversity of microorganisms. We will cross-sectionally study occupational exposure to bioaerosols and the immunome. In EXIMIOUS, 100 workers from 7–10 waste-sorting plants will have exposure measured personally and provide blood samples at the end of the working day for analyses.

Workers exposed to birds and fungi: In EXIMIOUS, 100 workers from the Urban Pest Control and Surveillance Service from Barcelona, Spain—with a broad range of exposure levels to avian and fungal antigens—will supply blood samples for analyses with speci c emphasis on their degree of sensitization to speci c antigens relative to the potential for risk of hypersensitivity pneumonitis (HP).

Workers exposed to mineral dust and organic solvent: This Romanian study population will include 100 miners with exposure to mineral dust such as silica and coal, 100 workers exposed to organic solvents in the paint industry, and 100 foundry workers from a metallurgic plant exposed to both mineral dusts (silica and metal particles) and organic solvents. These workers will be matched to a control group consisting of 200 of ce workers. Exposure will be assessed via direct

measurements complemented with questionnaires. Health status will be thoroughly evaluated by clinical examination, chest X-ray, and lung function test. Blood samples—and in selected cases bronchoalveolar lavage uid—will be collected to assess the immunome.

Disease populations

We will include populations of patients with ve types of immune-mediated diseases—both granulomatous and autoimmune diseases. The following study populations will be included in the project: (1) 100 HP patients, recruited from

with high-speed acquisition, up to 28 uorochrome-tagged antibodies on a single cell can be analyzed, providing the effective quanti cation of multiple immune populations, including rare populations and their activation status, from a single vial of blood. Using this highly sensitive tool, extensive cell immune pro ling can be carried out, theoretically for more than 1500 different cell subsets within the same patient, covering most of the major leukocyte subsets. The approach is robust and stable and scalable to the level of thousands of individuals. This approach combines the advantages of generating multiparameter analyses with those of high-throughput analysis (allowing the screening of 20–50 patients per day). Immune-cell pro les can be analyzed from either peripheral blood mononuclear cells (PBMCs) from the patient or directly from tissue after cell isolation. In addition, the cellular data will be complemented by plasma cytokine detection using multiplex assay with electroluminescence detection (MSD) detecting more than 50 parameters. While blood analysis can reliably identify systemic impact on the immune system, organ-speci c disease might display a unique local immune signature. Therefore, we also aim at investigating immune cells from tissues in some prospective cohorts for targeted exposed organs when such samples are available.

Deep immune profiling of innate immune cells

To map the innate immune system, we will use cytometry by time of ight (CyTOF) mass spectrometry. CyTOF is an advanced, high-dimensional platform (40–45 simultaneous markers in one tube), allowing larger antibody panels and less blood volume than ow cytometry. This method will be used for the detection of functional immune-cell pro les in selected whole blood samples from the population studies allowing in-depth immune pro ling with an innate focus, i.e., the classi cation of cell subtypes and their activation status, and the simultaneous detection of functional markers like intracellular cytokines, signaling pathways and proliferation. Combined with unsupervised mathematical algorithms, CyTOF is a powerful tool to explore and investigate the immune system with single-cell resolution and to identify new combinations of characteristics that are easily overlooked in traditional, supervised analyses. Complementary in vitro exposures in a whole blood model will be performed to identify exposure-induced immune cell pro les and to support causal interferences.

Multidimensional analysis of immunological parameters

Given the ultra-high parameter nature of the data generated by both ow cytometry and CyTOF, a traditional analysis based on manual gating is not feasible. The introduction of automated analysis will allow the quanti cation of thousands of immune parameters and the ability to normalize tech le blood modeea1s-ria

of exposures and immune system. We now have the ability to holistically explore and integrate the human immunome, using new systems-level technologies that combine multiparameter data from high-throughput sources. It is only very recently that the first systems-immunology studies have been published with respect to healthy humans,[5] demonstrating its tremendous potential. By integrating this "immunomics" in a multi-omics approach—including (functional) single-cell proteomics, genomics, epigenetics, transcriptomics, proteomics, and secretomics—we will gain a multi-dimensional insight into the different omics layers,[35] the flow of information and complex interactions.

The methodology of discovering immune fingerprints that are associated with immune-related disease has been successfully used by researchers in our consortium to identify the immuno-

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