

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

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Abstract: Immune-mediated, noncommunicable diseases—such as autoimmune and inflammatory diseases—are chronic disorders, in which the interaction between environmental exposures and the immune system plays an important role. The prevalence and societal costs of these diseases are rising in the European Union. The EXIMIOUS consortium—gathering experts in immunology, toxicology, occupational health, clinical medicine, exposure science, epidemiology, bioinformatics, and sensor development—will study eleven European study populations, covering the entire lifespan, including prenatal life. Innovative ways of characterizing and quantifying the exposome will be combined with high-dimensional immunophenotyping and -profiling platforms to map the immune effects (immunome) induced by the exposome. We will use two main approaches that “meet in the middle”—one starting from the exposome, the other starting from health effects. Novel bioinformatics tools, based on systems immunology and machine learning, will be used to integrate and analyze these large datasets to identify immune fingerprints that reflect a person’s lifetime exposome or that are early predictors of disease. This will allow researchers, policymakers, and clinicians to grasp the impact of the exposome on the immune system at the level of individuals and populations.

Key Words: External exposome; Immunome; Immune-mediated diseases; Multi-omics.

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 influences.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 specific, 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-specific, 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 confirmed this link between specific (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} In addition to “well-defined” autoimmune disorders, exposure has been linked to features that suggest autoimmunity—such as Raynaud’s phenomenon or the presence of autoantibodies, immune complexes, or excess production of immunoglobulin—but without the full clinical features of an autoimmune disease. Moreover, suffering from one autoimmune disorder increases the risk of developing additional autoimmune diseases (*polyautoimmunity*). There is clearly a strong need to assess the complexity of the immune effects and identify the major environmental drivers and their integrated effects.

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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 (five, Table 1). Furthermore, we will study immune-mediated diseases as a variety of disease phenotypes resulting from different combinations of gene–environment interactions, where some combinations of genes and environmental exposures can lead to certain disease phenotypes, while others might not (see Figure 1). These diseases can be considered to constitute only the “tip of the iceberg” of a spectrum of immune effects—the *immunome*. Mapping these immune effects will allow us to detect the early immune effects of exposure.^{11,19,20} Our consortium—gathering experts in immunology, toxicology, clinical medicine, exposure science, epidemiology, bioinformatics, and sensor development—will bring about a new way of assessing the human exposome by linking ways of characterizing and quantifying multiple and combined environmental and occupational exposures with high-dimensional immunophenotyping

and -profiling platforms to map the early immune effects induced by these exposures (*immunomics*).

Project description

General methodology

By combining several study populations (<https://www.eximious-h2020.eu/cohorts/>)—covering the entire lifespan, including prenatal life and occupational settings—we will study the role of the immunome as an intermediate factor between exposome and disease. We will use two main approaches that ‘meet in the middle’²¹—one starting from the exposome, the other starting from health effects (Figure 2). In general population, birth and occupational study populations, we first determine the exposome and then advance to search for immune fingerprints that reflect exposure. In the disease cohorts, we start from health effects, and next, we search for immune fingerprints reflecting both exposure and disease. By mapping the immunome from both directions, we aim to disentangle immune signatures reflecting a person’s lifetime exposure and immune signatures reflecting exposure-induced disease. To analyze these data, new approaches will be developed using systems immunology and machine learning.

Which populations are included in the project?

General population and birth cohorts

LifeLines cohort study²² is a large, three-generation prospective study and biobank in the Netherlands, starting in 2006 with at least 10 years of follow-up. It aims to understand healthy aging in the 21st century. A nested case-control approach will be applied that guarantees the prospective dimension of the study samples. We will select approximately 300 patients diagnosed with rheumatoid arthritis, 175 with systemic lupus erythematosus, 350 with type I diabetes, and 500 matched controls. To make use of the advantages of the nested design, we will perform both exposure measurements (urinary black carbon) and blood immune fingerprinting in samples that were biobanked at baseline to predict disease outcomes in alignment with temporal relations.

ENVIRonAGE (ENVIRonmental influence ON AGEing in early life) birth cohort²³ includes at the moment more than 2000 mother-child pairs in Belgium, starting in 2012 and currently still recruiting, of which more than 460 children and their mothers have been followed up between the child’s age of 4 and 6. EXIMIOUS will re-invite 150 children at 8–10 years of age and their mothers. Urine and blood were and will be collected from mother and child (at birth, at 4–5 and 8–10 years) alongside questionnaires, tests of cognitive functioning, and micro- and macrovascular phenotypes to assess their cardiovascular function. Cytokine measures in umbilical cord blood collected at delivery ($n = 750$) will be studied in the context of both prenatal and postnatal exposures including both the external exposome (green space and residential air pollution models) and measures of the internal exposome, such as carbon load in cord blood and the children’s urine. In blood from one hundred fifty 4–5-year-old children and 150 mothers entering the follow-up study, the immunome will be assessed.

DOC*X cohort²⁴ is an extraordinary cohort including the entire Danish population, for the study of working life and health. Entirely register-based, it includes life history of residents working at least 1 year in 1970+1976–2017, i.e., approximately 6.4 million people. Validated period-specific Job Exposure Matrixes (JEMs) list levels of exposure—such as noise, UV-radiation, dust (organic/inorganic), workload, stress, and different chemicals—for each occupational group. Hospital diagnoses (ICD8/10) are retrieved from the nationwide Danish National Patient Register.

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S.V. is the legal representative of STEVOH BV. STEVOH BV has been appointed as Managing director of the non-profit organisation BeCOH. S.V. works for the Nickel Institute, a global association of primary nickel producers. S.V. also works as external industrial hygiene consultant for many companies and associations in the context of the NEPSI, European Network for Silica. The views expressed are entirely those of the authors and do not necessarily reflect the Nickel Institute or NEPSI policy. All remaining authors declare that they have no conflicts of interest with regard to the content of this report.

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Table 1.
Overview of study populations included in the EXIMIOUS project

	Study Population	Time course			
		Prenatal	0–18 years	18–65 years	> 65 years
General population and birth cohorts	The LifeLines Cohort				
	ENVIRonAGE				
Occupational	DOC*X				
	DOC*X Generation				
	Waste workers				
Disease	Park workers				
	Workers exposed to mineral dust and organic solvents				
	Sarcoidosis				
	Hypersensitivity pneumonitis				
	Systemic Sclerosis				
	Systemic Lupus Erythematosus				
	Rheumatoid Arthritis				

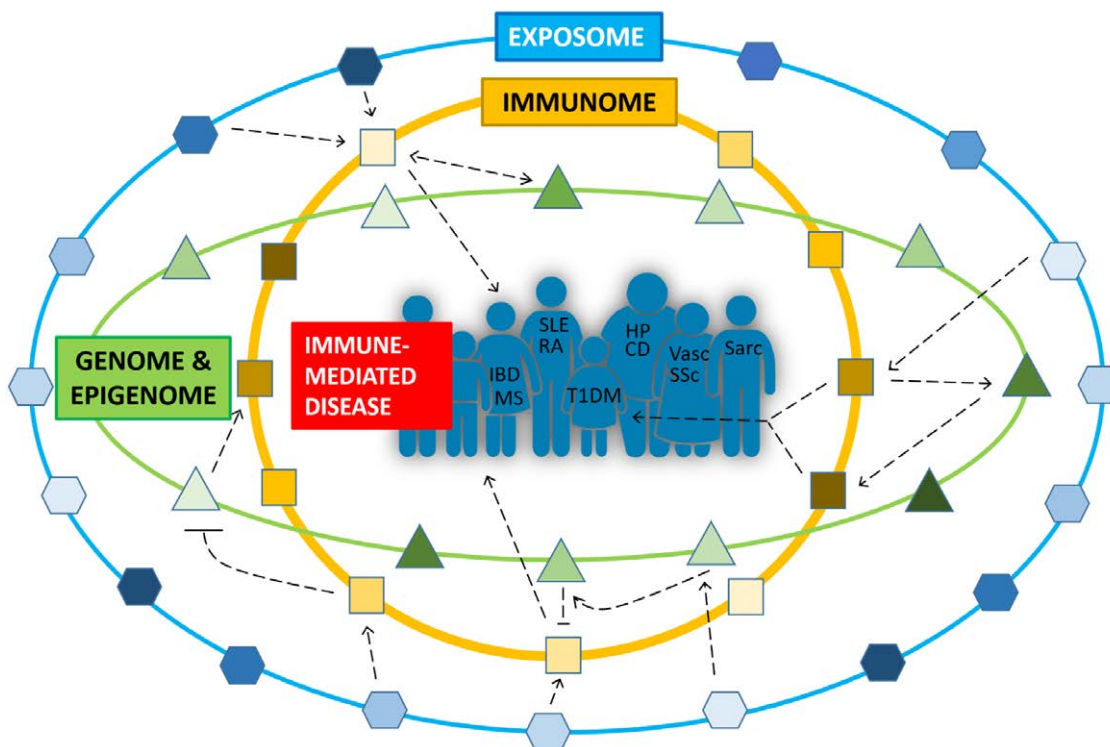


Figure 1. Exposome-genome-immunome interactions and immune-mediated disease [adapted from Gourley et al.²⁰]. Some combinations of environmental exposures and immune responses induce certain disease phenotypes (depending on genomic background), while other combinations might have no effect or could be protective. CD, celiac disease; HP, hypersensitivity pneumonitis; IBD, inflammatory bowel disease; MS, multiple sclerosis; RA, rheumatoid arthritis; Sarc, sarcoidosis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; T1DM, diabetes type 1; Vasc, vasculitis.

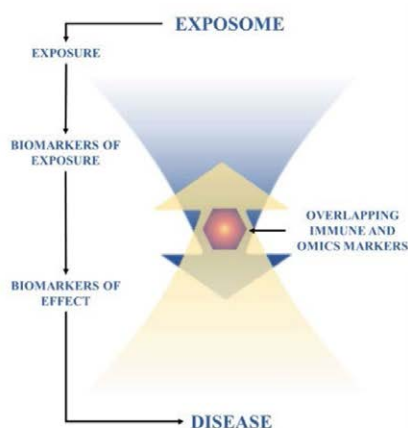
DOC*X Generation includes the children of all DOC*X members with their birth and health outcomes (>1.2 million children), with follow-up of up to 38 years of age. In the EXIMIOUS project, DOC*X and Generation will be updated until 2018. For this cohort, we will model air-pollution exposure at the individual 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 specific emphasis on their degree of sensitization to specific 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 office workers. Exposure will be assessed via direct



FIRST APPROACH: STARTING FROM THE EXPOSOME

We will begin with cohorts that cover the entire lifespan: general and birth cohorts (LifeLines, DOC*X and DOC*X Generation, ENVIRONAGE) and occupational cohorts (park workers, paint factory workers, miners, metallurgy workers, waste handlers and administrative workers).

SECOND APPROACH: STARTING FROM THE DISEASE

In this approach, we start from cohorts of people that have potentially exposure-related, immune-mediated diseases, like systemic sclerosis (SSc), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), sarcoidosis and hypersensitivity pneumonitis (HP).

Figure 2. The Eximious methodology: two approaches that meet in the middle.²¹

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 fluid—will be collected to assess the immunome.

Disease populations

We will include populations of patients with five types of immune-mediated diseases—both granulomatous and auto-immune diseases. The following study populations will be included in the project: (1) 100 HP patients, recruited from the pulmonary fibrosis clinic in Vall d'Hebron University Hospital (Barcelona, Spain); (2) 100 patients with newly diagnosed sarcoidosis recruited at the sarcoidosis clinic in the University Hospitals of Leuven (KU Leuven) Belgium, (3) 100 patients with systemic lupus erythematosus recruited at the Cliniques universitaires Saint-Luc (UCL), Brussels, Belgium, (4) 100 patients with rheumatoid arthritis (UCL), and (5) 200 patients with systemic sclerosis (KU Leuven and UCL). Also, 200 individuals without immune-mediated diseases will be recruited from the clinics for cardiovascular, abdominal, urological, oncologic, or orthopedic conditions (KU Leuven and UCL).

What will be measured?

1. Exposome

Exploiting existing data

Several of the EXIMIOUS cohorts already include data on exposure that are hitherto unexplored in relation to immune-related health effects. By exploiting and combining these data, we will make maximum use of previous research efforts, enabling us to incorporate data on psychosocial factors, physical activity, endotoxins, organic dust, mineral dust, noise, wood dust, diesel exhaust, volatile organic compounds (VOCs), fumes, vapors, pesticides, metals, food, noise, air pollution, indoor air quality, biomarkers of exposure—such as particles, black carbon, solvents, and metabolites of chemical products in different biological samples (blood, urine, placental tissue)—and several omics. Information about the prenatal exposome is also available in the birth cohorts (ENVIRONAGE, DOC*X Generation).

Mapping the exposome

The EXIMIOUS consortium includes a network of laboratories that are able to measure a huge range of components by

standardized and validated external or internal measurements. To map the exposome several of these “classic” collection strategies will be setup and combined. An overview of the most common is given in Table 2.

Modeling exposure

When data on (determinants of) occupational exposure are available, exposure will be modeled using existing and newly developed occupational exposure assessment tools. In disease cohorts, where accessing the workplace of the participant is difficult, exposure will be estimated based on a combination of interviews, job-exposure matrices, and expert assessment. To assess residential ambient air pollution (PM₁₀, PM_{2.5}, NO₂), established local and European land use and dispersion models are used.²⁵

Measuring external occupational exposures

Exposure assessments will be done at the workplaces, according to the exposure gaps recognized in the modeling. Internationally elaborated and established methods, e.g., EN and ISO standards will be used for the exposure assessments of airborne pollutants, such as volatile and semivolatile compounds, metals and metalloids, diesel particulate matter, and respirable crystalline silica. Furthermore, organic dusts will be assessed, including allergens, micro-organisms, microplastics, and natural polymers. Skin patches and wipe samples will be used to evaluate dermal and surface exposure. Most methods are validated for a certain exposure route (inhalation and skin) and for single substances (e.g. silica) or groups of substances (e.g., metals and metalloids). The development of new and affordable techniques for qualitative and/or quantitative determination of mixed exposures will require duplicate or parallel sampling.

Exposure to microplastics

Microplastics are considered a global concern in environmental and occupational contamination. While most focus is on the aquatic environment,²⁶ much less attention is paid to the presence of plastics as airborne particles. Methodologies for the characterization of microplastics in the environment are still under development. Often a combination of methods is used, including Fourier transform infra-red (FTIR), thermogravimetric analysis, eventually combined with FTIR, and gel permeation chromatography.⁹ One of the most powerful techniques is the combination of analytical pyrolysis with GC-MS (Pyr-GC-MS). This method, now under development by one of our consortium members (BeCOH), only requires a small amount of sample

Table 2.
Overview of environmental sampling strategies

Parameter	External samples			Biomonitoring	
	Additional information	Standard / Guidelines	Matrix	Parameter	Technique
AIR			Blood	Solvent screening	SHS-GC-MS
Aerosol	inhalable, thoracic and respirable fractions; extra thoracic and tracheobronchial	ISO 7708 ISO 13138.		Halogenated POPs (PCBs, HCB, PDBEs, DDT/ DDD/DDE)	GC-MS/MS
Gasses and volatiles	Ambient Indoor atmospheres	ISO/TC 146/SC 3 ISO/TC 146/SC 6		Musks (cosmetics)	GC-MS/MS
Metals and metalloids		EN 13890 ISO 15202 ISO 30011		Parabens (cosmetics)	LC-MS/MS
Diesel particulate matter (DPM)	Organic Carbon (OC), Elemental Carbon (EC) OR Total Carbon (TC).	EN 14530	Urine	ETU (carbamate pesticide metabolite)	LC-MS/MS
Crystalline silica		ISO 16258-1 & 16258-2 ISO 19087		Solvent screening	SHS-GC-MS
Volatile organic compounds (VOCs)		ISO 16000–6		Phenol & cresol (benzene, toluene exposure)	SPME-GC-MS
Semi-volatile compounds such as	polyaromatic hydrocarbons (PAHs)	ISO 12884		Muconic acid, hippuric acid, mandelic acid methylhippuric acid, (BTX exposure)	GC-MS
Aldehydes	formaldehyde, acetaldehyde, acrolein	ISO 16000–3		Paraben metabolite: hydroxybenzoic acid	LC-MS/MS
SKIN				Hydroxy-PAH (PAH metabolites)	GC-MS/MS
Aerosols and volatiles	Skin patch Wipe sample	CEN/TR 15278 ISO/TR 14294		Bisphenol-A	GC-MS/MS
Metalloids on skin		ASTM D7822		3-PBA, FPBA (pyrethroid pesticide metabolites)	GC-MS/MS

GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; SHS, static headspace; POP, persistent organic pollutant.

and can be used to differentiate polymer types, and allows both qualitative and quantitative analysis.

Exposure to environmental microbiome

Exposure to the environmental microbiome will be assessed based on the genomic DNA (gDNA) from dust samples collected from the occupational study sites, along with appropriate positive and negative (mock communities, extraction- and nontemplate PCR controls), processed on an automated nucleic acid purification platform (Roche MagNA Pure 96 System) as described before.²⁷ Absolute bacterial abundance will be measured by quantitative real-time PCR, using both 16SrRNA and genus-specific primers.²⁸ Comprehensive molecular characterization of samples will be performed using the Illumina MiSeq NGS platform targeting the 16S rRNA marker gene as previously described.²⁹ This method allows detection of difficult to culture and nonculturable taxa and enhances the ability to identify bacteria in samples with low abundance. Amplicon libraries will be grouped into operational taxonomic units (OTUs) on the basis of sequence similarity cut-offs, typically 90–97% similarity against publicly available 16S rRNA gene reference genomes, followed by classification to a particular taxonomic level (from kingdom to genera). Estimates of diversity (richness, Shannon-Wiener Index and Simpson Index) will be calculated and taxon-based community structures will be compared to determine if there are changes in community structure with time and differences dependent on exposure. Ecological relationships between bacteria will also be analyzed to determine the main drivers of community variance and bacterial co-occurrence. Analysis of metagenomic data generated from NGS platform will also be performed to evaluate the abundance of genes encoding antimicrobial resistance.

Internal biomarkers of ambient air pollution

There is a high societal need for adequate internal exposure markers allowing reduction of exposure misclassification. In this project, we will use an internal marker of black carbon as recently developed within an ERC proof of concept grant (INCALO). This biomarker is based on the label-free and

biocompatible detection of carbon-based particles under femtosecond pulsed illumination.³⁰ The integration of this method in EXIMIOUS will allow us to make a direct linkage of black carbon with observed health effects and immunome profiles.

Hyperspectral imaging (HSI)

The spectral properties of airborne substances captured on sampling substrates can be used to develop techniques for a direct identification of the sample composition. Specific sample characteristics might be related to exposure profiles in certain industries and to potential health effects. For example, the toxicity of quartz is related to the time when the particle is produced (freshness) and reactions that take place at the surface of the particle,³¹ which can be identified by spectral infrared (IR) analysis.³² The hyperspectral imaging technique (imec's Snapscan system) will be used to identify substances on sampling substrates taken in parallel during exposure assessment (skin exposure and airborne exposure). A correlation study will be performed between the validated methods and Snapscan results. Until now, determining the levels and composition of dust in homes or workplaces is time-consuming, requiring complex logistics and analytical techniques. When validated, HSI could offer many new opportunities for mapping the exposome, because it can identify the composition of samples quickly and cheaply (see Figure 3).

2. Immunome

The immune system can be viewed as a human exposure sensor, as it changes throughout a person's lifetime, mainly in response to environmental triggers.^{5,33} We will use various high-dimensional platforms to map the individual's immune system by assessing both function and phenotype (*immunomics*). It is one of the fundamentals of the project that an insight into the immunome can bring us complementary information about the exposome.

Deep immune profiling of adaptive immune cells

We will use an advanced immunophenotyping platform allowing in-depth immune profiling of the adaptive immune system. Using an innovative flow-cytometry analyzer (BD FACSymphony)



Figure 3. (A) imec Snapscan VNIR hyperspectral camera with LS150+ bands in 470–900nm. (B) Compact hyperspectral imaging (HSI) camera. (C) HSI image with robust classification of different ore oxide minerals with various colors and different chemical composition

with high-speed acquisition, up to 28 fluorochrome-tagged antibodies on a single cell can be analyzed, providing the effective quantification 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 profiling 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 profiles 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 electrochemiluminescence detection (MSD) detecting more than 50 parameters. While blood analysis can reliably identify systemic impact on the immune system, organ-specific 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 flight (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 flow cytometry. This method will be used for the detection of functional immune-cell profiles in selected whole blood samples from the population studies allowing in-depth immune profiling with an innate focus, i.e., the classification 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 profiles and to support causal interferences.

Multidimensional analysis of immunological parameters

Given the ultra-high parameter nature of the data generated by both flow cytometry and CyTOF, a traditional analysis based on manual gating is not feasible. The introduction of automated analysis will allow the quantification of thousands of immune parameters and the ability to normalize technical variation introduced via longitudinal sampling. Additionally, data-driven (unsupervised) analyses will be performed to identify cell populations and immune cell fingerprints suitable as effect or disease biomarkers.

3. Multi-omics

Transcriptomic, (epi)genomics, and secretomics will be carried out to complement the immunome data and to complete our understanding of the immune effects of the exposome (see Figure 4). First, to identify epigenetic changes associated with chemical exposome and/or health outcomes, an epigenome-wide association study (EWAS) will be conducted. Genome-wide DNA methylation will be measured using the cost-effective Illumina Infinium Human Methylation EPIC array. In addition, to the analyses of genome-wide DNA methylation, we will study plasma/serum miRNA profiles in the participants. Bioinformatics and statistical tools—both open-source and commercially available software—will be used for the identification of differentially methylated regions (DMRs), together with their associated regulatory networks. Furthermore, a genome-wide association study (GWAS) will be undertaken. Participants will be genotyped for 700,078 variants using the Infinium® HTS assay on Global Screening Array bead-chips (Illumina). Genotype calling will be done using GenomeStudio V2011.1 software. Next, telomere length—as an integrated exposome marker—will be measured using a validated qPCR protocol. Finally, we will characterize the cellular secretome by performing proteomic analysis of extracellular fluids (plasma, sputum).

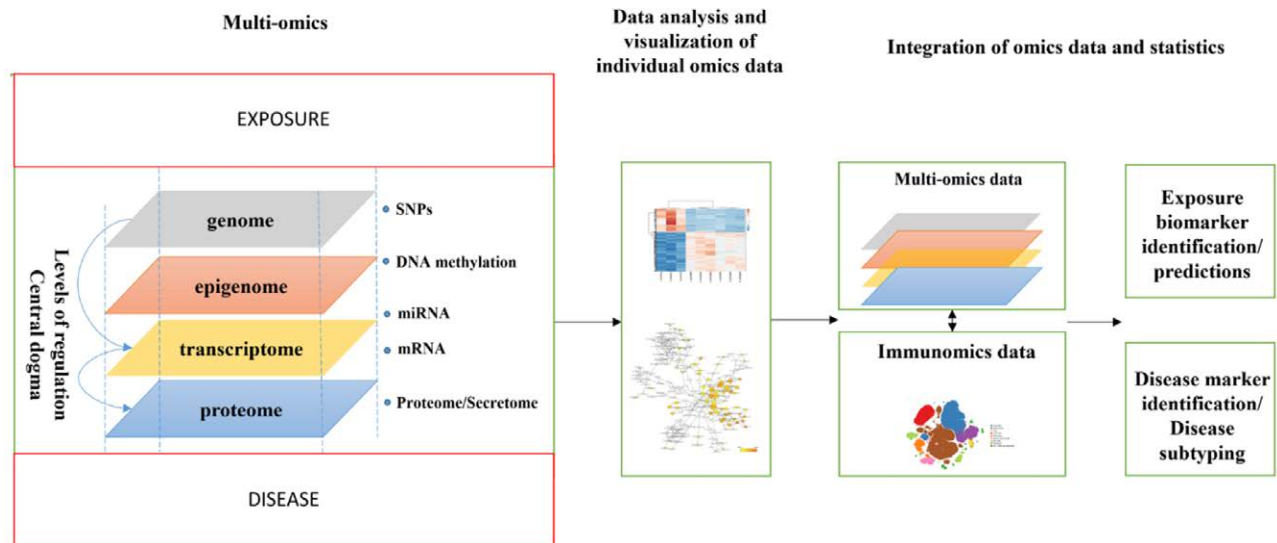


Figure 4. Schematic representation of the “multi-omics” approach—a unique combination of immunomics with other omics. mRNA, messenger RNA; miRNA, microRNA; SNP, single nucleotide polymorphism.

4. Combining it all—statistics and systems immunology

We will use a combination of statistical approaches and bioinformatics tools, based on systems immunology and machine learning, to integrate and analyze the extensive datasets created by high-throughput platforms measuring the internal and external exposome, immunological outcomes (immunome), other omics, and clinical data.

Exposures and immune-related diseases in large cohorts

The Danish DOC*X cohort (with employment, exposure, and health data for more than 6 million people and 1.2 million of their children) and the Lifelines cohort contain a wealth of clinical and socio-economic data that remains unexplored in terms of the exposome and immunome. Neural networks will be used to identify causality between (combinations of) exposures, at different time points in life (adult, prenatal) and the risk of developing autoimmune disease. Hypotheses will be tested using prospective study designs that will guide the selection of exposures to monitor in the other cohorts. Thanks to the size of the DOC*X cohort, we can be able to discover new—including rare—associations between exposome and immune effects, which are impossible to identify in smaller databases.

Systems Immunology—machine-learning analysis of the immune fingerprints associated with exposure and disease

Systems Biology/Systems Immunology is an emerging scientific field that has seen major advances in the ability to combine and analyze large datasets coming from new, high-dimensional, immune-profiling platforms. To identify immune-parameter patterns associated with occupational exposure and/or autoimmune disease, we will consider a pan cohort multi-class classification model. As input for the classification model, we will use the outcome variables with all the immune parameters measured (immunome), treated as one high-dimensional data point per individual in each cohort. The primary aim of this approach will be to identify, which individual immunological variables are driven by the exposome and/or disease. In addition, this will greatly reduce the dimensionality of the immunological data for the secondary analysis, which focuses on identifying individual exposures that drive the immune variation, thus making this analysis statistically tractable. Examples of our approach can

be found at DOI:10.1038/s41467-021-23126-8 and 10.1016/j.envint.2020.106283.

Multiple exposure analyses, meet-in-the-middle cross-omics approach, and GWAS to integrate exposome, immunome, and clinical disease data

As a complementary approach, we will apply multiple exposure models in which correlation structures between the immunome, epigenetics—miRNA and DNA methylation targets and telomere length—and multiple exposures will be visualized per cohort via ideograms to search for candidate biomarkers, whose levels are affected by exposures. We will identify the principal axes of immunome signals in response to exposures on the path towards disease among different omic levels by path and mediation analyses to assess the role of immune parameters as intermediate variables (mediators) between exposures and disease. Finally, a GWAS analysis will be performed to identify genotype-dependent effects of environmental exposures. Studying genetic variants that are known to disrupt exposure metabolism (single genes or complex pathways) has been demonstrated to be a promising strategy for identifying disease-related variants that interact with exposure.³⁴

Strengths and limitations

To date, studies in the field of immune-related health effects have focused on one or a few exposures and studied a narrow set of autoantibodies and/or diseases. These studies have shown that the concept of exposure-related immune-mediated health effects is sound but needs to be broadened. In the EXIMIOUS project, we will try to broaden the scope (1) from one agent to a better understanding of the influence of whole exposome, (2) from selected immune effects to an in-depth while broad map of the immune system (immunome), and (3) from one disease to a broad spectrum of immune-mediated diseases. This will allow us to better understand the contribution of different environmental risk factors to the immune-mediated health effects, leading to a sound prioritization of targeted preventative actions.

EXIMIOUS will include a range of cohorts, which all have their strengths and limitations. While the DOC*X and Lifelines cohorts encompass large populations including a range of available register data, most other cohorts have more limited numbers of participants, but focus on the in-depth study

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 immunological parameters that (statistically) predict juvenile idiopathic arthritis (JIA) disease status and disease activity.³⁶ The pioneering study involved 85 children with JIA and 43 control children. A machine-learning approach, using random forests and 10-fold cross-validation, trained an algorithm with 91% accuracy in predicting JIA status. The analysis of the dominant parameters in the model identified the frequency of the iNKT cells as the single parameter with the strongest association with the disease status. This demonstrates the utility and efficacy of our immunomics approach to identify biomarkers with diagnostic potential in relatively simple tests.

Our approach might also lead to improvements in immune-related disease prediction, as for the LifeLines cohort we will analyze bio-banked blood samples from participants before they had developed disease.

Conclusion

EXIMIOUS will develop a novel approach to assess the human exposome by combining innovative ways of characterizing and quantifying multiple environmental exposures (exposome) and mapping exposure-induced immune effects (immunome and immune diseases). Novel bioinformatics tools will be developed, using systems biology, artificial intelligence, and machine learning, to combine and analyze the huge datasets linking the exposome, immunome, and other omics data with clinical and socio-economic data. By exploring the entire pathway from exposome, to immune fingerprint, to disease during a person's lifetime (including prenatal exposure) we will better understand the mechanisms that lead to exposure-related immune effects at different stages of life and pinpoint the most relevant exposures and people at risk to direct specific preventative actions and policies at the individual, group, and population levels, and so contribute to better healthcare.

The innovation potential of EXIMIOUS does not lie only in the separate innovations of the various disciplines but in the fact that with this consortium, we have brought together the expertise from fields that simply did not interact before, enabling us to make a leap forward in mapping the exposome, as we will broaden its scope to include the immunome.

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