Advanced Non-animal Models in Biomedical Research

Autoimmune diseases

Executive Summary
This Executive Summary describes a study conducted by the JRC’s EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) to identify current and emerging non-animal models and methods being used for biomedical research related to cardiovascular diseases.


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The immune system defends our body from external threats, such as viruses, bacteria or allergens, and is able to distinguish these from our own cells. Sometimes however, it can falsely recognise constituent elements such as joint tissues or skin as being foreign and thus attacks them as a result. This can leads to a wide variety of autoimmune diseases.

One prominent example of autoimmune disease is type 1 diabetes, in which the immune system destroys the cells that produce insulin in the pancreas.

There are more than 100 identified illnesses with an autoimmune origin (Box 1). They are generally considered to be relatively uncommon but in fact they affect 3–5% of the worldwide population (1), and their contribution to mortality and morbidity are significant.

Coping with complexity: the need for reliable experimental models

The mechanisms behind autoimmune diseases are complex and not completely understood, mainly because they likely result from interactions between genetic and environmental factors.

Animal models – mainly mouse and rat – are used to study disease mechanisms and test therapeutic strategies. However, there are a number of important biological differences between human and rodent immune systems that often limit extrapolation from animal models to patients.

Human-based models have the potential to more faithfully represent the clinical features of human autoimmune diseases, offering better insight into human-specific biology and pathology. Thus the JRC’s EU Reference Laboratory for alternative to animal testing (EURL ECVAM) carried out this study to identify and characterise the state of the art of human-

based models in the field of biomedical research for autoimmune diseases.

The study covers approaches that use cells and tissues cultured in the laboratory (in vitro methods), computer modelling and simulation (in silico methods) or cells and tissues explanted from patients (ex vivo methods).

**Advanced models for human-relevant research**

The EURL ECVAM study analysed the scientific literature from January 2014 to March 2019 and identified 183 models that used advanced non-animal models for autoimmune disease research. These models are mainly used to study five autoimmune diseases: rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, type 1 diabetes and psoriasis.

The majority of them are based on in vitro methods (Figure 2; Box 2) that mainly use human primary cells. The use of these type of cells is important to understand the genetic, molecular and cellular factors that contribute to human pathogenesis. This is because human primary cells come from body tissues and therefore retain the functional and morphological characteristics of their donor.
Box 1. Autoimmune diseases

There are over 100 autoimmune diseases that affect the body in a wide range of ways. They are frequently classified into organ-specific or non-organ-specific disorders. In organ-specific disorders, the autoimmune process is directed mostly against one organ, for example the thyroid gland or pancreas. In non-organ-specific disorders, autoimmune reactions happen throughout the body.

Figure 1. Autoimmune diseases: target organs, symptoms and risk factors.

Some autoimmune disorders are well known, such as:

- **Type 1 diabetes**, a condition in which the immune system damages the insulin-producing cells in the pancreas;
- **Multiple sclerosis**, a nervous system disease where the immune system attacks the myelin sheath, which is the outer layer of nerve cells in the brain and spinal cord;
- **Lupus**, a disease that damages different areas of the body including joints, skin and organs;
- **Rheumatoid arthritis**, where the immune system attacks the lining of the joints.
- **Thyroid diseases** including Graves’ disease, where the body makes too much thyroid hormone (hyperthyroidism), and Hashimoto’s thyroiditis, where it doesn’t make enough (hypothyroidism);
- **Psoriasis**, a condition marked by thick, scaly patches of skin.

Others conditions are rare and difficult to diagnose. With unusual autoimmune diseases, patients may suffer years before getting a proper diagnosis.

The exact causes of autoimmune disorders are not known. The risk factors seem to include genetics, environment, sex hormones and lifestyle. Also infections from bacteria, viruses or toxins, and treatment with some drugs, may play a role in triggering an autoimmune process in someone who already has a genetic (inherited) predisposition to develop such a disorder.
they provide a valuable means to gain new insights and test hypotheses, particularly when used in conjunction with clinical observations and *in vitro* models.

**The knowledge base**

This study has produced a unique and highly curated knowledge base that contains detailed descriptions of 183 advanced non-animal models being used for autoimmune disease research.

It is freely available to download from the EURL ECVAM Collection in the JRC Data Catalogue (2), together with a JRC Technical report (3) that describes the review methodology and presents the main findings (see Box 3).

This unique knowledge base can serve the needs of multiple stakeholders:

- **Researchers** can identify models and methods which can be adapted and applied to tackle their own research questions;
- **Educators** can provide the latest information on the state-of-the-art to their students;
- **Funding bodies** can consider trends, identify impactful research avenues and target promising areas for investment;
- **Project evaluation committees** can ensure that project proposers have properly considered the use of non-animal models and methods in their research proposals;
- **National Contact Points** and **National Committees** (4) can ensure proper knowledge sharing on non-animal methods within Member State networks and organisations involved in biomedical research using animals.

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2. [https://europa.eu/WDjTjH](https://europa.eu/WDjTjH)


4. As referred to in Directive 2010/63/EU for the protection of animals used for scientific purposes.
Box 2. **Advanced in vitro models**

The EURL ECVAM study found that **organoids**, which are miniaturised 3D representations of an organ, are commonly used to study autoimmune diabetes (see Figure 3). Either human embryonic or induced pluripotent stem cells are used to generate cells producing insulin in the form of organoids.

In a few cases, researchers also used simple 3D models generated by spontaneous cell aggregation, called **spheroids**, to study different aspects of type 1 diabetes. Both types of 3D cultures – organoids and spheroids – improve cell differentiation and maturation in order to obtain functional cells with consistent insulin production.

**Organ slices** are an **ex vivo** model used to study autoimmune diseases affecting skin. These skin slices have 3D spatial features for studying cells in their natural microenvironment in a more physiological manner.

They are often cultivated together with immune cells to analyse cell-tissue interactions, immune activation, and underlying biological mechanisms. Moreover, this type of model can incorporate different types of immune cells to better mimic particular aspects of human physiology and pathogenesis under investigation.

*Figure 3.* Human pancreatic tissue from non-diabetic (A) and type 1 diabetes organ donors (B) grown as organoids. They expand with a cauliflower-like appearance; some larger cyst-like structures are also present. © Loomans, C. et al., 2018, *Stem Cell Reports*, 10, 712–724, doi:10.1016/j.stemcr.2018.02.005.
Findings of this study can also inform aspects of policy making regarding the protection of animals used for scientific purposes, setting of research priorities to progress the development and uptake of non-animal methods, and the promotion of modern human-relevant scientific approaches to combat autoimmune diseases.

Finally, this knowledge base provides a means to explore the strengths and limitations of both animal and non-animal models used in biomedical research, to stimulate healthy scientific debate, to challenge mind-sets, and to pave the way for doing better and more predictive science. Thus, the knowledge base helps to bridge across methods and disciplines in the biosciences (5) to improve biomedical research for the ultimate benefit of patients and society.


Box 3. Knowledge base of advanced non-animal models

This study is a part of a series that EURL ECVAM has carried out to review available and emerging non-animal models being used for research in seven disease areas. Details on the published studies are available on the EURL ECVAM website.

In this study around 3,000 peer-reviewed publications on autoimmune diseases were initially retrieved and screened for representative papers describing innovative and promising advanced non-animal models.

An important outcome of this study is a highly curated knowledge base containing detailed descriptions of 183 non-animal models being used for autoimmune disease research. It is easily downloadable as a spreadsheet file from the EURL ECVAM collection in the JRC Data Catalogue.

This knowledge base is complemented with a Technical report that provides an in-depth analysis of the models identified and of the review methodology used (Figure 4).
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