The Experimental Induction of Autoimmune Disease

By Craig Paardekooper

The basic principle

This is well-trodden ground in experimental immunology. The textbook description is the induction of experimental autoimmune disease by immunisation with self-antigens alongside an immunological adjuvant.

The method is simple enough to state in a sentence. You take a protein the body already makes, mix it with something that provokes the immune system, and inject the mixture. The provocateur is usually complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) or pertussis toxin (PTX). Given the right conditions, the immune system learns to attack the self-protein, and what you are left with is an autoimmune disease you made on purpose.

The parallel with experimentally induced food allergy is not a loose analogy. The immunological logic is the same in both cases: an antigen the body normally tolerates can become a target once it is presented in an inflammatory or immunostimulatory setting.

Myocarditis from cardiac myosin

The cleanest illustration of the principle comes from Kodama and colleagues in 1990, who described a new model of giant cell myocarditis in rats immunised with a cardiac myosin fraction (*Clinical Immunology and Immunopathology* 57(2), 250–262).

The ingredients were few: cardiac myosin, which is a self-protein taken from heart muscle; complete Freund's adjuvant; and Lewis rats. The emulsion went in by subcutaneous injection into the hind footpad.

What came back was severe inflammatory heart disease — myocardial necrosis, inflammatory cells pushing into the tissue, multinucleated giant cells, and a rise in antibodies against cardiac myosin itself.

That is the whole argument in one experiment. Immunise an animal against one of its own proteins in an adjuvant context, and you get a genuine organ-specific autoimmune disease.

The same model, with a second adjuvant

Afanasyeva and colleagues returned to the model in 2001, reporting that experimental autoimmune myocarditis in A/J mice is an interleukin-4-dependent disease with a Th2 phenotype (*American Journal of Pathology* 159(1), 193–203; open access). Cardiac myosin immunisation produced histologically confirmed myocarditis, myosin-specific antibodies, inflammatory infiltration of the myocardium, and a measurable role for IL-4 in how the disease developed.

But there is a complication, and it matters. The protocol used two immunostimulants, not one, and by two different routes:

Component	Route
Cardiac myosin (self-antigen)	Subcutaneous
Complete Freund's adjuvant	Subcutaneous, emulsified with the myosin
Pertussis toxin	Intraperitoneal

The mice were immunised subcutaneously on days 0 and 7, with the intraperitoneal pertussis toxin given on day 0. The toxin was a deliberate addition, not an incidental one, and the experiment cannot be read properly without accounting for it.

Why use both? Because their effects overlap without being identical. CFA is killed mycobacteria suspended in an oil emulsion; it drives local inflammation, pulls in and activates antigen-presenting cells, sharpens antigen presentation, and pushes the response towards strong cellular immunity. Pertussis toxin is a bacterial immunomodulatory protein, and in these models it alters immune-cell signalling, shifts inflammatory responses, changes how immune cells traffic and how permeable the vasculature is, and generally makes autoimmune pathology easier to establish. It is often called a second adjuvant, or an adjuvant-enhancing agent, in the encephalomyelitis and myocarditis literature.

So what does the study actually establish? That cardiac myosin immunisation, in an adjuvant-rich inflammatory environment and with systemic pertussis toxin on board, can induce autoimmune myocarditis. What it does not establish, on its own, is that CFA alone would have been sufficient, that PTX was necessary, or that myosin and CFA without the toxin would have produced the same disease. The authors' controls and mechanistic work do say a good deal about the immune pathways involved; they simply do not disentangle the two adjuvants.

Two further sources are worth having alongside it. Wang and colleagues (1999) characterised murine autoimmune myocarditis induced by self and foreign cardiac myosin (*Autoimmunity* 31, 151–162), which speaks to how antigen-specific the response really is. There is also a

methodological review covering autoimmune myocarditis, valvulitis and cardiomyopathy, which goes through the use of cardiac myosin, CFA and PTX in these experiments.

My own classification of the Afanasyeva experiment would be: self-antigen plus multiple immunostimulatory agents. Cardiac myosin as the self-antigen, CFA as the primary adjuvant, pertussis toxin as an additional immunostimulant; antigen and adjuvant subcutaneous, the extra immunostimulant intraperitoneal; outcome, autoimmune myocarditis. That is not a clean demonstration of autoimmune disease produced by a self-protein and a single immunogenic ingredient.

Which leaves the question worth asking: are there animal experiments showing autoimmune disease after a self-protein combined with just one immunostimulatory substance, with no pertussis toxin and no other immune-enhancing agent in the protocol? That distinction is what makes the comparison with the food-allergy experiments meaningful.

Thyroiditis from thyroglobulin

Maron and colleagues (1983) worked with thyroglobulin, the normal self-protein made by the thyroid gland, in a study of a T lymphocyte line that either produces or vaccinates against autoimmune thyroiditis in mice (*Journal of Immunology* 131(5), 2316–2322).

Immunising with thyroglobulin in adjuvant generated autoreactive T lymphocytes. Those thyroglobulin-reactive T-cell lines could then be transferred into other animals, where they caused thyroiditis. And the disease ran independently of circulating antibodies against thyroglobulin.

The interest here is in the mechanism rather than the outcome. An immune response raised against a self-protein can produce pathogenic cellular immunity — not merely autoantibodies, but cells that do the damage themselves.

Encephalomyelitis from myelin proteins

Experimental autoimmune encephalomyelitis (EAE) is the best known of all these models. The self-antigens in play are myelin basic protein, myelin oligodendrocyte glycoprotein and proteolipid protein.

The paper most relevant here is Hofstetter and colleagues (2002), on how pertussis toxin modulates the immune response to neuroantigens injected in incomplete Freund's adjuvant — inducing Th1 cells and EAE in the presence of high frequencies of Th2 cells (*Journal of Immunology* 169(1), 117–125). Pertussis toxin substantially reshaped the response to self-neuroantigens and promoted the development of disease, which is a direct demonstration that a bacterial immunostimulant can steer immunity against a self-protein towards pathology.

Two features of the protocol deserve to be kept separate. Freund's adjuvant supplied the inflammatory environment in which the neuroantigen was presented to the immune system; pertussis toxin was given separately and acted as an additional immunomodulatory stimulus that helped the disease along. And note that the antigen here was emulsified in *incomplete* Freund's adjuvant, not complete, with the toxin administered on its own.

The experiment therefore shows that a self-protein can become the target of a pathogenic immune response when immunostimulatory agents are present. It does not show that a single immunostimulant combined with the self-protein would have been enough.

What adjuvant alone can do

One paper deals with the adjuvant by itself, given intraperitoneally. Fawcett and colleagues (1990) reported the induction of humoral manifestations of autoimmunity following intraperitoneal injection of complete Freund's adjuvant in mice (*Autoimmunity* 6(4), 249–256).

CFA on its own, by that route, was enough to produce antinuclear antibodies, antibodies against cytoplasmic antigens, a rise in circulating immune complexes, and rheumatoid factor in some of the animals. What it did not produce was overt arthritis.

That gap is the point of the paper. An immunostimulatory substance can generate the serological markers of autoimmunity without going on to cause a defined autoimmune disease, and the two should not be run together.

The evidence so far, and the question of route

Disease	Self-protein	Immunogenic substance	Route
Myocarditis	Cardiac myosin	CFA	Subcutaneous
Myocarditis	Cardiac myosin	CFA + pertussis toxin	Subcutaneous + IP
Thyroiditis	Thyroglobulin	Adjuvant	Historical immunisation model
Encephalomyelitis	Myelin proteins	Freund's adjuvant + pertussis toxin	Usually SC/ID + IP or IV
Humoral autoimmunity	Endogenous self-antigens	CFA	Intraperitoneal

Set out like this, the literature points towards a sharper question than the one it usually answers. Can a self-protein and an immunostimulatory substance, given together by the *same* systemic

route — intravenous or intraperitoneal — break tolerance and produce a clinically demonstrable autoimmune disease?

Three experimental situations have to be held apart here, because they are not equivalent and the distinction is routinely passed over:

1. Self-protein and adjuvant given together intravenously.
2. Self-protein and adjuvant given together intraperitoneally.
3. Self-protein by one route, with the immunostimulant given separately by IV or IP injection.

Look again at what the classical studies actually did with the route:

Study	Self-antigen	Adjuvant	Route of antigen/adjuvant	Additional agent
Kodama (1990)	Cardiac myosin	CFA	SC (hind footpad)	Pertussis vaccine, IV
Afanasyeva (2001)	Cardiac myosin	CFA	SC	Pertussis toxin, IP
Hofstetter (2002)	Neuroantigen	IFA	SC	Pertussis toxin, separately

In every case the antigen–adjuvant emulsion went in subcutaneously, not intramuscularly, intravenously or intraperitoneally. These experiments establish that a self-antigen combined with an immunological adjuvant can induce autoimmune disease. They say nothing about whether delivering that same combination by the IM, IV or IP route produces the same outcome.

The useful next step is therefore narrow: find the animal studies where the self-protein and the immunostimulant were administered together by the same IM, IV or IP route, rather than adding to the much larger subcutaneous literature. That is also what would make the comparison with the food-allergy work worth drawing.

Studies where CFA worked without pertussis

There are experiments in which a self-antigen was combined with CFA alone, no pertussis toxin anywhere in the protocol, and autoimmune disease followed. That is a meaningful step away from the two-adjuvant studies above. Three examples stand out, one cardiac and two in other organs.

Uveitis from retinal S-antigen. Hirose and colleagues (1991) immunised several rat strains with retinal soluble antigen in complete Freund's adjuvant, as part of work separating MHC

from non-MHC gene effects in experimental autoimmune uveitis (*Clinical and Experimental Immunology* 86, 419–425). Lewis rats developed uveitis with no pertussis involved at all. Other strains, under identical conditions, did not — and additional intravenous *Bordetella pertussis* was what allowed those resistant strains to develop disease. So CFA plus a self-antigen can be sufficient, provided the animal is genetically susceptible, and genetic susceptibility turns out to carry a great deal of the weight.

Thyroiditis from thyroid extract. Vladutiu and Rose (1972) used thyroid extract, containing thyroglobulin, emulsified in sonicated CFA (*Clinical and Experimental Immunology* 11, 245–254). They produced thyroid-specific autoantibodies, inflammatory infiltration of the gland, and experimental autoimmune thyroiditis. Subcutaneous immunisation worked better than intradermal. Pertussis vaccine was not needed — and, interestingly, when it was included it reduced disease incidence.

Myocarditis from cardiac myosin. Kodama's 1990 paper, above, reports Lewis rats immunised with human cardiac myosin in CFA developing severe myocarditis with extensive infiltration and giant cells, and the published abstract describes the myosin–CFA immunisation without naming pertussis toxin as a required component. I would keep this on the list as a promising CFA-only example while checking the complete original methods before classifying it that way for certain.

A fourth study is worth adding: a 1996 report on the immunosuppression of thyroiditis, in which immunisation with human thyroglobulin in CFA produced histological thyroiditis, anti-thyroglobulin antibodies and antigen-specific lymphocyte proliferation — evidence that CFA-assisted immunisation against a self-protein can raise both humoral and cellular autoimmune responses.

Study	Self-antigen	CFA	PTX required?	Disease
Hirose (1991)	Retinal S-antigen	Yes	No, in susceptible rats	Uveitis
Vladutiu & Rose (1972)	Thyroid extract	Yes	No	Thyroiditis
Kodama (1990)	Cardiac myosin	Yes	Not reported as required in abstract	Myocarditis
Immunosuppression of thyroiditis (1996)	Human thyroglobulin	Yes	Not reported as required	Thyroiditis

Taken together, these make a good case that CFA can serve as the principal immunostimulatory adjuvant in experimental autoimmune disease, without pertussis toxin being necessary. The

qualification is the same one as before: every clearly documented example uses subcutaneous administration of the antigen–CFA emulsion.

One more point on strain genetics. Silver and colleagues (1999) later showed that whether pertussis is required in experimental autoimmune uveitis depends on the animal's genetic background: B10.RIII mice developed disease after exposure to retinal antigen without PTX, while B10.A mice did not.

The distinction still missing from the picture is a CFA-only study using intraperitoneal or intravenous administration of the antigen–adjuvant combination. I would treat that as a separate category outright, since the subcutaneous studies cannot establish that the same thing happens through systemic injection.

Does the adjuvant have to be mixed with the antigen?

In principle, no. An immunostimulant can create an inflammatory environment near a tissue, change the behaviour of local antigen-presenting cells, or affect how autoreactive lymphocytes are activated and where they travel — none of which requires it to be physically emulsified with the self-antigen.

But three situations get conflated here, and the evidence for them is not equally strong:

4. Physical mixing, where self-antigen and adjuvant are combined in one emulsion.
5. Separate administration within the same anatomical region, where the two are unmixed but may act on the same local immune environment.
6. Local immunostimulation near an organ, where the adjuvant acts in the vicinity of a tissue and may influence responses to self-antigens available there.

Bystander activation. A 2017 PLOS ONE study of bystander activation of irrelevant CD4+ T cells following antigen-specific vaccination found that intramuscular CFA produced local immune activation in draining lymph nodes, and that transferred CD4+ T cells with no specificity for the immunising antigen were activated locally too. The qualification is important: this is local activation of unrelated T cells, not the induction of a defined autoimmune disease. It does support the broader idea that immunostimulation reaches beyond the particular antigen physically bound up with the adjuvant.

Pertussis toxin acting at a distance. Su and colleagues (2001) showed that PTX inhibits the induction of tissue-specific autoimmune disease by disrupting G protein-coupled signals (*Journal of Immunology* 167, 250–256). The value of this paper is that it cuts against the simple picture: PTX has profound systemic effects on immune-cell migration and on disease even when given separately from the antigen, and here it actually *suppressed* experimental autoimmune uveitis when administered at certain stages of the response. PTX is not merely an amplifier. What it does depends on timing and immunological context.

Co-exposure at a mucosal surface. Liu and colleagues (2024) described an intranasal sensitisation model of alopecia areata using pertussis toxin as adjuvant (*Frontiers in Immunology* 15, 1469424; open access). PTX and MOG peptide were given intranasally together, and a proportion of animals developed alopecia areata, with inflammatory changes around the hair follicles, signs of systemic immune activation, and MOG-specific lymphocyte responses in the animals receiving both agents. Notably, they did not get conventional EAE, which had been the intended outcome. This is evidence of autoimmune pathology after mucosal co-exposure. It is not evidence that PTX placed near an organ, independently of the self-antigen, is sufficient to cause organ-specific autoimmunity.

Experimental situation	**Evidence**
Self-antigen physically mixed with CFA	Strong evidence for experimental autoimmunity
Self-antigen and PTX administered separately	Strong evidence that PTX can modify systemic immune responses
CFA causing local bystander activation	Demonstrated experimentally
PTX and self-antigen co-localised at a mucosal surface	Emerging experimental evidence
CFA or PTX near an organ, unmixed with its self-antigen, causing organ-specific autoimmune disease	Not established by the papers discussed here

The biology does allow for it. The immune system does not require the adjuvant to be physically attached to the antigen, and antigen-presenting cells, inflammatory mediators and lymphatic drainage can all connect events happening in different places. But proximity is not causation, and local inflammation does not reliably end in autoimmune disease.

Where this leaves things

Two hypotheses are worth naming, because the literature tends to blur them.

The **local inflammatory hypothesis** holds that an immunostimulant produces inflammation near an organ, which may be enough to facilitate an immune response against self-antigens released from that tissue. The **antigen-specific sensitisation hypothesis** holds that the self-antigen must itself be presented in an immunostimulatory context before a pathogenic antigen-specific response can arise.

The 2017 bystander activation study and the 2024 intranasal PTX/MOG study are useful starting points for testing the two against each other. Neither one establishes that simply placing CFA or PTX near an organ induces autoimmune disease.

What would move this forward is a focused look at experimental studies of local adjuvant exposure and organ-specific autoimmunity, keeping four outcomes strictly apart: local inflammation, bystander T-cell activation, autoantibody production, and histologically confirmed autoimmune disease. Collapsing those four into a single claim is what most of the confusion in this area rests on.

A Note on Aluminium

Aluminium is unambiguously an immune stimulant. That is its function and nobody disputes it. Its adjuvant effect has been ascribed to its particulate nature and depot formation, with the mechanism more recently shown to include activation of inflammasomes.

And there are models where alum plus a self-antigen does establish disease. The clearest is experimental autoimmune prostatitis. Forty C57BL/6 mice were split into four groups — T2 peptide plus aluminium hydroxide, T2 alone, aluminium hydroxide alone, and control — and histopathology showed prostate lesions most severe in the group immunised with T2 plus aluminium hydroxide, with elevated plasma TNF-α and CRP and raised IL-1β in the prostate; the authors concluded that subcutaneous T2 in aluminium hydroxide successfully established the model. There is a parallel rat model using prostate extract with aluminium hydroxide injection. The design matters here: antigen alone and adjuvant alone were both controls, and neither produced the disease. That is structurally the same experiment as the CFA studies in your document. See https://urotoday.com/recent-abstracts/men-s-health/prostatitis/101083-establishment-of-experimental-autoimmune-prostatitis-model-by-t2-peptide-in-aluminium-hydroxide-adjuvant.html