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REVIEW ARTICLE

Do basophil activation tests help elucidate allergic reactions to the ingredients in COVID-19 vaccines?

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Abstract

The worldwide use of COVID-19 vaccines has shown that immediate allergic reactions to the ingredients are rare but should be clarified by means of an allergological work-up. This review aims to highlight the current state of knowledge and possible pathogenesis based on the literature published to date. In addition to recording a detailed history and performing skin tests, cellular tests (basophil activation or basophil histamine release test) by using the vaccines or modified compounds containing polyethylene glycol (PEG), rather than unmodified PEGs, have proven to be particularly helpful. Negative results with vaccines seem to indicate tolerance. Details of the performance of these cellular tests with different vaccines, PEGs of different molecular weights, other ingredients of the vaccines, as well as other PEGylated drugs, and the results in the context of COVID-19 vaccination of various working groups worldwide are summarized.

KEYWORDS
basophil activation test, basophil histamine release test, COVID-19 vaccines, mRNA vaccines, polyethylene glycol (PEG)

1 | INTRODUCTION

After the initial round of vaccination in December 2020 against COVID-19 in the UK, three cases of suspected anaphylaxis in connection with the vaccine were reported, and it was believed that immediate-type allergic reactions could be a common problem.[1] Meanwhile, the risk is measured to be 2.5-11/1,000,000 by vaccine safety programs. It must be assumed that some of the previously reported reactions were not anaphylactic, but vasovagal events and signs related to anxiety. It was shown that in the vast majority of patients reporting such reactions, a second vaccination was tolerated without any problems.[2] Nevertheless, in rare cases, there were clear indications of a vaccine-induced anaphylactic reaction. Anaphylaxis was confirmed in 0.027% of individuals who received the Pfizer-BioNTech vaccine (BNT-vaccine) and 0.023% of individuals who received the Moderna vaccine (M-vaccine).[3] Among the suspected triggers, ingredients of the vaccines, mainly PEGs but also polysorbate 80 and tromethamine, were blamed.[1,4] Potential mechanisms inducing anaphylaxis due to COVID-19 mRNA vaccines include contact system activation by nucleic acids, complement recognition of the vaccine-activating allergic effector cells, direct mast cell activation, and pre-existing antibody recognition of PEG.[5] The classical methods of allergological work-up include skin tests, determination of sIgE, and, in advanced diagnostics, cellular tests. The basophil histamine release test (BHRT) and basophil activation test (BAT) are established *in vitro* tests for this indication. The aim of

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this review is to investigate whether these tests are helpful in the work-up and investigation of immediate-type allergic reactions to COVID-19 vaccine ingredients.

2 | PRINCIPLE OF CELLULAR TESTS

For advanced diagnostics of immediate-type allergic reactions, cellular *in vitro* tests can be used; these predominantly prove the sensitization of basophils. The tests use the detection of mediators or cellular antigens that are measurable upon successful activation. Enriched blood leukocytes or whole blood is incubated with allergens or other triggers. The surface markers expressed after allergen stimulation or the mediators released by basophils usually serve as an indirect measure of cellular-bound specific IgE. However, IgE-independent stimuli also elicit basophil activation.

The tests are useful if problems arise in conventional diagnostics, either in interpretation (for example, in the case of contradictory results) or performance (for example, when skin tests for eczema or symptomatic dermographism are not feasible or evaluable). Furthermore, for rare allergens, the determination of sIgE antibodies may not be possible. In addition, provocation testing may not be feasible due to the pharmacological properties of drugs, the severity of the reported reaction, or ethical concerns (for example, risk of re-sensitization).[6,7]

3 | DETAILS OF THE METHODS

Cellular tests should be performed with fresh cells since a loss of activity can be expected after 4 h. However, storage of the cells for a maximum of 24 h is acceptable because EDTA blood is sufficiently stable and therefore shipping is possible. Depending on the test protocol, whole blood or enriched leukocyte suspensions can be used.[6]

Soluble and non-cytotoxic substances can be used as allergens. With regard to the PEG derivatives, vaccines and other drugs listed in Tables 1 and 2, there were no indications of toxicity due to the substances used. These substances should be used at different concentrations. In most studies, two to six concentrations were used; two studies used only one concentration.[8,9] A negative control and positive controls (IgE-dependent and/or IgE-independent stimuli) must be included. To rule out non-specific activation with particular allergens, non-sensitized control subjects should be tested. Both aspects were considered in most of the studies listed in Table 2. Basophils from approximately 5%–15% of cell donors cannot be activated after IgE-mediated stimulation (non-responders). In such cases, the tests are false negative.[6,7] In this overview, five non-responders have been reported.[10,11]

The basophil histamine release test (BHRT) established by Lichtenstein's group in the 1970s is based on the measurement of the preformed mediator histamine released from the granules of basophils. It can be measured spectrofluorometrically, enzymatically or radioimmunologically. Histamine release from individual samples is usually expressed as a percentage of the total histamine concentration or measured in ng/ml.[12]

In addition to the direct incubation of basophils with allergens, the incubation of serum from allergic patients with IgE-depleted donor basophils is also possible (passive sensitization of basophil granulocytes).[6,7] This method was applied to BHRT in two studies with the substances used here.[11,13]

Over the last decades, the use of the basophil activation test has increased compared to the BHRT due to the faster analysis by flow cytometry, and histamine being unstable and difficult to reliably detect. The determination of basophil activation is based on flow cytometric detection of activation markers on basophils. For IgE-mediated reactions, the markers CD63 and CD203c have been used. In the presented studies, CD63 was more often used as an activation marker than CD203c. CD63, a component of granule membranes, is not a basophil-specific marker and is expressed in other blood cells. Therefore, further labelling is required to identify basophils. Possible markers include anti-CCR3, anti-IgE, anti-CRTH2 (excluding CD3-positive cells), CD203c and anti-CD123 (excluding HLA-DR-positive cells). This was the main difference between the tests used. CD203c is a basophil-specific marker that is constitutively expressed. Because the use of different identification markers has little influence on the results, this is not explicitly listed in Tables 1 and 2. CD203c and CD63 are upregulated after IgE receptor aggregation, but have partially different metabolic pathways and follow different kinetics. Interleukin-3 potentiates allergen-induced CD63 expression without itself upregulating CD63, whereas it increases CD203c expression even in the absence of allergen ('priming' marker). The results of basophil activation tests are usually expressed as percent activated basophils, and occasionally as mean fluorescence intensity (MFI). Threshold values or stimulation indices are given for the individual allergens in the commercially available tests; otherwise, they must be calculated using receiver operator characteristic (ROC) curves.[6,7]

4 | INGREDIENTS OF COVID-19 VACCINES USED IN CELLULAR TESTS

COVID-19 mRNA vaccines are lipid nanoparticles formulated to encapsulate mRNA transcripts. The formulation components include cationic and ionizable lipids with three parts (headgroup, linker and tails), sterols, phospholipids and PEG-anchored lipids, which define their properties.[14]

The mRNA vaccines contain mRNA (mRNA encoding the viral spike (S) glycoprotein of SARS-CoV-2), buffer constituents (tromethamine in the M-vaccine) and lipids. Among them, there are PEGylated lipids (BNT-vaccine: 2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; M-vaccine: 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol 2000 (PEG2000-DMG)), ionizable lipids (BNT-vaccine: [(4-hydroxybutyl)azanediyl)]bis(hexane-6,1-diyl) bis(2-hexyldecanoate); M-vaccine: SM-102 (proprietary)), neutral lipids (1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol).[15]

The adenovirus vector-based COVID-19 vaccines contain the chimpanzee adenovirus vector including the gene of the glycoprotein spike

(S) antigen of SARS-CoV-2, L-histidine, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80 (E 433), ethanol, sucrose, sodium chloride and disodium edetate (Vaxzevria, AstraZeneca; AZ-vaccine), or recombinant, replication-incompetent adenovirus type 26 encoding a stabilized variant of the SARS-CoV-2 spike (S) protein, citric acid monohydrate, trisodium citrate dihydrate, ethanol, 2-hydroxypropyl-β-cyclodextrin (HBCD), polysorbate 80 and sodium chloride (Janssen COVID-19 Vaccine, Johnson and Johnson; J-vaccine).[15,16]

The inactivated Chinese SARS-CoV-2 vaccine (CoronaVac, Sinovac; S-vaccine) contains inactivated SARS-CoV-2 virus, aluminium hydroxide, disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate monohydrate and sodium chloride.[17]

An overview of allergies and COVID-19 vaccines including possible triggers can be found in an ENDA/EAACI position paper.[18] Among the ingredients in COVID-19 vaccines, PEGs are deemed to be the possible culprit of anaphylactic reactions. Cross-reactivity with polysorbate 80 has been discussed.[1,3,4,19] For this reason, these compounds in particular were investigated in cellular tests. Among other excipients with allergenic potential in COVID-19 vaccines, disodium EDTA and trometamol are mentioned, but they are not among the tested substances listed in Table 2.[15]

5 | CELLULAR TESTS IN PATIENTS ALLERGIC TO PEG OR POLYSORBATE BEFORE THE INTRODUCTION OF COVID-19 VACCINES (UNTIL 2019)

5.1 | Patients and controls

Before the introduction of the COVID-19 vaccine, cellular tests (either BHRT or BAT) were performed in 10 patients with PEG or polysorbate 80 allergy (BHRT: $n = 2$; BAT: $n = 8$) with positive results in five cases.[13,20,21,22,23,24,25,26] Only in two manuscripts, data about controls with negative results were published.[13,24]

5.2 | Positive results with components

Positive results were found with the culprit substances containing PEG 3350, PEG 4000, PEG 6000 and PEG 8000, as well as those with PEG 1500, PEG 3350, PEG 4000 and PEG 6000. In one case, BAT with polysorbate 80 was also positive. For details see Table 1.

5.3 | Concentrations of components used

Unfortunately, details of the concentrations used were not always provided. However, information about PEG 3350 used at 10% and PEG 6000 at 100%,[13,20] PEG 3000 at 100%,[20] PEG 6000 at 1:100 000 dilution,[23] PEG 4000 at 1%,[24] as well as drugs containing PEG 4000 in different dilutions[25,26] can be found in the cases with positive results. In another case, PEG 1500 and PEG 6000 were used at concentrations from 5 ng/ml to 500.000 ng/ml and PEG 4000 at concentrations ranging from 1.5 ng/ml to 500 mg/ml.[26] Polysorbate 80 was positive at 0.02 mg/ml in one case.[24]

5.4 | Basophil activation (%) or histamine release (ng/ml) results

%CD63 or %CD203c activation ranged from 14.9% to 75.9%, and maximum histamine release was 25 ng/ml. For details see Table 1.

5.5 | Immunological mechanisms

Detailed studies involving preincubation with monovalent ethylene glycol, diethylene glycol and omalizumab, which bound IgE antibodies, and a passive-positive BHRT demonstrated an IgE-dependent mechanism in one case.[13]

6 | CELLULAR TESTS WITH COVID-19 VACCINES, PEGS, POLYSORBATE AND OTHER RELATED COMPOUNDS DURING THE PERIOD OF COVID-19 VACCINES (SINCE 2020)

In 2021, significantly more cellular tests and components were used in individuals with planned COVID-19 vaccinations and suspected allergy to the ingredients of the vaccines to clarify suspected allergic reactions after COVID-19 vaccinations. In summary, 31 positive results were reported (Table 2).

6.1 | Patients and controls

Published data from over 100 patients are available to date, with BAT used predominantly. Eighteen patients with a diagnosed PEG allergy and 91 patients with suspected allergic reactions to COVID-19 vaccination were assessed. In addition, results from approximately 50 controls were available. For details see Table 2.

6.2 | Used COVID-19 vaccines and other components

BNT-vaccine, M-vaccine, AZ-vaccine, J-vaccine and S-vaccine were used.

Polyethylene glycols with different molecular weights (PEG 200, 400, 600, 2000, 3000, 3350, 4000, 6000 and 20,000), 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol 2000 (DMG-PEG 2000), 2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-PEG 2000) and PEGylated doxorubicin 2000–3500 were tested.

Other components used were polysorbate 80, polysorbate 20, poloxamer 407 and SARS-CoV-2 spike peptides.

6.3 | Concentrations of vaccines and components used

6.3.1 | Vaccines

In most studies, BNT-vaccine was used in the BAT at four concentrations ranging from 0.05 μg/ml to 10 μg/ml,[27] 0.01 μg/ml to 10 μg/ml[28] or 0.18 μg/ml to 22.7 μg/ml.[26] In two studies, only one concentration for mRNA vaccines was used (0.007 μg/μl and 1 μl, respectively).[8,9] Best positive results were found for concentrations around 10 μg/ml. In BHRT, the BNT-vaccine was used at six concentrations.[29]

M-vaccine was used in only two studies with a concentration of 0.007 μg/μl[8] or a range from 0.36 μg/ml to 45.45 μg/ml.[26] In BHRT, M-vaccine was used at six concentrations.[29]

AZ-vaccine was used in a series of dilutions ranging from 1:2000 to 1:10[27] or from undiluted to 1:125 (division by 4.4 to calculate the final concentration).[26] In BHRT, AZ-vaccine was used at six concentrations.[29]

The J-vaccine was used in only one study, ranging from undiluted to 1:125 dilution (division by 4.4 to calculate the final concentration).[26]

S-vaccine was used in dilutions of 1:10 and 1:100.[30]

6.3.2 | Unmodified PEG 2000 and modified compounds containing PEG 2000

Polyethylene glycol 2000 as a component of the mRNA vaccines was used most often, sometimes at only one concentration and sometimes at up to six concentrations. The overall range was between 0.036 mg/ml and 15 mg/ml (single studies: 0.05 μg/ml to 5 mg/ml[27]; 0.1 μg/ml to 100 μg/ml[28]; 0.036 mg/ml to 15 mg/ml[26]).

DMG-PEG 2000 as a component of the M-vaccine was used in the BAT at one concentration (1 μg/μl)[8] or at three concentrations (0.0728 μg/ml to 1.82 μg/ml),[26] and in the BHRT at six concentrations.[29]

ALC-PEG 2000 as a component of the BNT-vaccine was only used in the BHRT at six concentrations.[29]

PEGylated Doxorubicin 2000–3500 was used in two studies, concentration ranging from 1 μg/ml to 10 μg/ml.[10,27]

6.3.3 | Other PEGs

Polyethylene glycol 200, PEG 400 and PEG 600 were used at a concentration of 5 mg/ml in one study[27] in the BAT, PEG 300 and PEG 3000 at concentrations of 0.0001 mg/ml to 10 mg/ml in the BHRT.[11] PEG 3350 was used in the BAT at concentrations ranging from 0.6 mg/ml to 15 mg/ml,[26] PEG 4000 at concentrations from 0.08 mg/ml to 4 mg/ml[31] or 0.036 mg/ml to 15 mg/ml,[26] and PEG 6000 at concentrations from 0.6 mg/ml to 15 mg/ml.[26] PEG 3350, PEG 6000 and PEG 20,000 were used at concentrations of 0.0001 mg/ml to 10 mg/ml in the BHRT.[11]

6.3.4 | Polysorbate and others

Polysorbate 80 was used in two studies at concentrations of 1 μg/μl[8] and 22.7 mg/ml (0.23%) and 2.3 mg/ml (0.023%).[26] Polysorbate 20 and Poloxamer 407 were only used in the BHRT.[11,29] SARS-CoV-2 spike peptides were used at dilutions of 1:100 and 1:1000.[30]

6.4 | Basophil activation (%) or histamine release (ng/ml) results

Vaccines: In clearly diagnosed PEG-allergic patients, maximal CD63% activation in one study with three patients was 51%, 64.2% and 82.1%,[27] and 21.3%, 34.4% and 37.2% in another study with the BNT-vaccine.[26] In one study with BNT-vaccine, the results in patients supposed to be allergic were expressed in stimulation indices (SI) with values of 2.88, 3.1, 3.19 and 4.79.[28]

With the M-vaccine in clearly diagnosed PEG patients, values of 16.1%, 20.5%, 34.8% and 41.8% were found.[26]

In mRNA-vaccine-allergic patients, a study with 13 patients revealed values from 9% to 56%, but it was not indicated which of the two mRNA vaccines was used in each individual case.[8] In another case, the value for the vaccine in the BAT was 23.3%.[9]

A study of one vaccine-reactive patient with values >15 ng/ml histamine release using BNT-vaccine, M-vaccine and AZ-vaccine was published.[29]

Polyethylene glycol 2000 and derivatives: PEGylated doxorubicin was found to be positive in three PEG-allergic patients with maximal values of 22.3%, 31.6% and 35.4%.[27] In 12 out of 13 mRNA-vaccine suspected allergic patients, DMG-PEG 2000 induced values from 10% to 73%.[8] PEG 2000 SI of 3.1. and 4.57 were found in two BNT-vaccine suspected allergic patients.[28] For PEG 4000, maximal CD63% activations were 14.79% and 16.2% (one patient with reaction to BNT-vaccine and one PEG-allergic patient, respectively) and 35.8% for PEG 6000 in one PEG-allergic case.[26,31]

In the BHRT, four PEG patients were positive for other PEGs, one for PEG 20 000, one for PEG 3000, 6000 and 20 000 (additionally also for poloxamer 407), one for PEG 3350 and 6000 and one for PEG 3000 and 6000.[11]

Polysorbate 80 was negative in 19 PEG-confirmed patients.[8,11,26]

The details can be found in Table 2, with a summary of the most important results in Table 3.

TABLE 1 Cellular test results in patients allergic to PEG or polysorbate before the introduction of COVID-19 vaccines (until 2019)

Test (activation marker)	Tested substances	Concentration(s)/dilution(s)	Patients/ controls for cellular tests	Threshold	Results	Patient(s) (re) vaccinated	Comments	Literature (order according to time of publication)
BAT^{0} (CD203c)	PEG 4000 PEG 6000 PEG 400	Different dilutions, among others 1:100.000	PEG patient: $n = 1$ No controls	Not published	**Positive:** **PEG 4000: 50% activation** **PEG 6000** Negative: PEG 400	n.a.	Systemic reaction after SPT with culprit drug	Bommarito et al., 2011[23]
BAT^{0}	PEG 3350	Not published	PEG patient: $n = 1$ No controls	Not published	Negative	n.a.	Anaphylactic shock after intradermal injection of macrogol 3350	Borderé et al., 2012[22]
BAT^{0} (CD203c)	Poly 80	Not clearly published (1:10–1:1000 dilution?)	Poly 80-patient: $n = 1$ No controls	Not published	Negative	n.a.	Systemic reaction after SPT with polysorbate 80	Badiu et al., 2012[21]
BHRT^{Ref} (direct and indirect)	Culprit drugs (with PEG 3350 and 6000) PEG 3350 PEG 6000 Ethylene glycol Diethylene glycol	Six concentrations ranging from <0.01 mg/ml to <100 mg/ml dependent on the substance PEG 3350: 1:10 PEG 6000: 1:1	PEG patient: $n = 1$ Control: $n = 1$	>5 ng of histamine release per ml blood	**Patient—Positive (up to 25 ng/ml histamine release):** **Culprit drugs** **PEG 3350** **PEG 6000** Negative: Ethylene glycol Diethylene Glycol Control: Negative to all substances	n.a.	Indirect BHRT with culprit drug (with PEG 3350) and PEG 6000 positive; preincubation with ethylene glycol, diethylene glycol, or Omalizumab abolished PEG-mediated HR	Wenande et al., 2013, 2016[13,36]
BHRT^{0}	PEG 3000 PEG 6000	100%	PEG patient: $n = 1$ No controls	Not published	Negative	n.a.	Convincing history for PEG and Poly 80	Wenande et al., 2015, 2016[20,36]
BAT^{0}	PEG 400 PEG 4000 PEG 6000 Poly 80	Not published	PEG patient: $n = 1$ No controls	Not published	Negative	n.a.	Oral provocation test with culprit drug positive	Badiu et al., 2015[37]

TABLE 1 (Continued)

Test (activation marker)	Tested substances	Concentration(s)/dilution(s)	Patients/ controls for cellular tests	Threshold	Results	Patient(s) (re) vaccinated	Comments	Literature (order according to time of publication)
BAT^{Detail} (CD63)	Culprit and tested drugs (with PEG 3350, 4000, 6000, 8000, Poloxamer 407) PEG 3350 PEG 4000 Poly 80	Drugs: not published (decreasing concentrations) PEG 4000: 1% Poly 80: 0.02 mg/ml, 0.002 mg/ml	PEG patient: $n = 1$ Controls (number not published)	Not published	**Patient—Positive:** **Culprit and tested drugs: 14.9%–37.3% activation** **PEG 4000: 17.1% activation** **Poly 80 at 0.02 mg/ml** Controls: Negative to all substances	n.a.	Convincing history for PEG and PEG derivatives (Poly 80, Poloxamer 407)	Jover Cerdá et al., 2019[24]
BAT^{0} (CD63)	Culprit drugs (with PEG 4000)	1:1000	PEG patient: $n = 1$ No controls	Not published	**Positive:** **Culprit drugs: 36.7% and 53.13% activation**	n.a.	Convincing history	Giangrande et al., 2019[25]
BAT^{Detail} (CD63)	PEG 4000 (manufacturer 1): $n = 2$ Culprit drugs (with PEG 400 and 4000) PEG 300 PEG 1500 PEG 4000 (manufacturer 2) PEG 6000: $n = 1$	PEG 4000 (manufacturer 1): 0.005 mg/ml–500 mg/ml PEG 4000 (manufacturer 2): 1.5 ng/ml–910 ng/ml PEG 300, PEG 1500, PEG 6000: 5 ng/ml–500.000 ng/ml Culprit drugs: 6 dilutions (1:4–1:12,500)	PEG patients: $n = 2$ No controls	Difference to baseline ≥10% CD63^{+} basophils	**Patient 1—Positive:** **Culprit drugs: max. activation: 44.3% and 75.9%,** **PEG 1500: max. activation 44.3%** **PEG 4000 (manufacturer 1): max. activation: 75.9%,** **PEG 4000 (manufacturer 2): max. activation: 70.2%,** **PEG 6000: max. activation: 54.3%** Negative: PEG 300. Patient 2: Negative: PEG 4000 (manufacturer 1)	Patient 1: not vaccinated Patient 2: n.a.	Oral provocation tests with PEG-containing drugs positive BAT-positive patient: BAT performed twice with PEG 4000 at two different time points with equivalent results, PEG 1500 and PEG 6000 also positive, but PEG 300 negative	Brockow et al., 2021 (Table 1)[26] and additional information by author JF

Abbreviations: 0, no detailed description of method given; BAT, basophil activation test; BHRT, basophil histamine release test; Detail, detailed description of method or commercially available test; HR, histamine release; max., maximal; n.a., not applicable; PEG, polyethylene glycol; Poly, polysorbate; Ref, reference for method mentioned; SPT, skin prick test.

Bold print indicate positive results.

TABLE 2 Cellular test results in patients with a supposed allergy to PEG or COVID-19 vaccines

Test (activation marker)	Tested substances	Concentration(s)/dilution(s)	Patients/controls for cellular tests	Threshold	Results	Patient(s) (re) vaccinated	Comments	Literature (order according to time of publication)
BAT^{Detail} (CD203c)	PEG 4000	6 concentrations (0.08 mg/ml–4 mg/ml)	BNT-vaccine suspected reactive patient: $n = 1$ Vaccine not reactive controls: $n = 5$	≥4% activation	**Patient—Positive:** **PEG 4000: 14.79% activation at 0.2 mg/ml, higher values at 0.1 mg/ml and 0.08 mg/ml compared to controls** Controls: Negative to all substances	No information about second vaccination	-	Restivo et al., 2021, April[31]
BAT^{Detail} (CD63)	BNT-vaccine AZ-vaccine PEG-Dox PEG 200 PEG 400 PEG 600 PEG 2000 PEG 6000	BNT-vaccine: 4 conc. (0.05 µg/ml–10 µg/ml) AZ-vaccine: 1:2000–1:10 dilution PEG-Dox: 1 µg/ml –10 µg/ml PEG 2000: 0.05 µg/ml–5 mg/ml PEG 200, PEG 400, PEG 600, PEG 6000: 5 mg/ml	PEG patients: $n = 3$ Vaccinated and unvaccinated controls: $n = 3$	Greater than 25% of that of the positive control (anti-IgE) corresponding to 14.9%, 14%, 18.3%	**Patients—Positive:** **BNT-vaccine: $n = 3$: max. activation: 51.0%, 64.2%, 82.1%** **PEG-Dox: $n = 3$: max. activation: 31.6%, 35.4%, 22.3%** Negative: AZ-vaccine, PEG 200, PEG 400, PEG 600, PEG 2000, PEG 6000 Controls: Negative to all substances	No information	Convincing history of PEG allergy	Troelnikov et al., 2021, April[27]
BHRT^{Ref}	PEG 300 PEG 3000 PEG 6000 PEG 20,000 Ethylene glycol Diethylene glycol Poly 80 Poloxamer 407	6 concentrations (0.0001 mg/ml to 10 mg/ml)	PEG patients: $n = 10$ (at two different time points) Controls: $n = 16$	>10% if found in 2 consecutive concentrations	**Patients—Positive:** **PEG 20 000: $n = 1$ patient** **PEG 3000, 6000, 20 000, Poloxamer 407: $n = 1$ patient** **PEG 3350, 6000: $n = 1$ patient (later negative)** **PEG 3000, 6000: $n = 1$ patient (later negative)** Non-releasers: $n = 4$ patients Negative: PEG 300, ethylene glycol, diethylene glycol, poly 80, poloxamer 407: $n = 10$ patients, 16 controls: negative to all substances	n.a.	Indirect BHRT positive: $n = 1$ Convincing history of PEG allergy	Bruusgaard-Mouritsen et al. 2021, May[11]

TABLE 2 (Continued)

Test (activation marker)	Tested substances	Concentration(s)/dilution(s)	Patients/controls for cellular tests	Threshold	Results	Patient(s) (re) vaccinated	Comments	Literature (order according to time of publication)
BHRT[Ref]	BNT-vaccine M-vaccine AZ-vaccine DMG-PEG 2000 ALC−0159- PEG 2000 PEG 2000 PEG 3000 PEG 3350 PEG 6000 PEG 20.000 Poly 80 Poly 20	6 concentrations	Total: $n = 61$ Vaccine suspected reactive patients described in detail: $n = 9$ No controls	Significantly positive: Bell-shaped curve with at least two positive values above baseline; marginally positive: Release above 15 ng/ml not fulfilling the criteria above	**Patients—Positive:** **BNT- and M-vaccine:** **$n = 1$ patient** Marginally positive: BNT-vaccine: $n = 1$ patient AZ-vaccine: $n = 1$ patient M-vaccine: $n = 1$ patient Negative: DMG-PEG 2000, ALC−0159-PEG 2000, PEG 2000, PEG 3000, PEG 3350, PEG 6000, PEG 20.000, Poly 80, Poly 20	4 patients described in detail successfully revaccinated 48 patients not described in detail successfully revaccinated	Oral challenge test to PEG 3350 positive in the patient positive in BHRT; marginally positive patient: COVID−19 infection 50 days before	Rasmussen et al. 2021, July[29]
BAT[Ref] (CD63)	mRNA-vaccines DMG-PEG 2000 Poly 80	mRNA-vaccines: 0.007 μg/μl DMG-PEG 2000: 1 μg/μl Poly 80: 1 μg/μl	mRNA-vaccine suspected reactive patients: $n = 13$ Vaccinated controls: $n = 3$	Positive response: ≥9% activation	**Patients—Positive:** **mRNA vaccines: $n = 13$: 11%, 29%, 21%, 39%, 67%, 23% 12%, 23%, 9%, 74%, 15%, 56%, 13%** **DMG-PEG 2000: $n = 12$: 22%, 22%, 14%, 73%, 21%, 14%, 25%, 11%, 17%, 14%, 61%, 10%** Negative: Poly 80: $n = 13$ Controls: Negative to all substances	No information	No PEG-IgE detected PEG-IgG in all tested individuals	Warren C et al. 2021, September[8]
BAT[Detail] (CD63)	BNT-vaccine PEG 2000	BNT-vaccine: 4 concentrations: 0.01 μg/ml–10 μg/ml PEG 2000: 4 concentrations: 0.1 μg/ml–100 μg/ml	Total: $n = 17$ Vaccine suspected allergic patients: $n = 6$ Controls: $n = 18$: Not vaccinated controls after COVID−19 infection: $n = 5$ Vaccinated controls after COVID−19 infection: $n = 5$ Vaccinated controls: $n = 4$ Not vaccinated controls: $n = 4$	Results calculated with SI (stimulation index) with a spontaneous activation around 2.5%. Cut-off points: 3 (PEG 2000: 100 μg/ml), 2 (10 μg/ml BNT-vaccine), 2.5 (1 μg/ml BNT-vaccine)	**Patients:-Positive:** **BNT-vaccine:** **$n = 4$ patients: SI: 3.1, 4.79, 3.19, 2.88** **$n = 5$ controls after COVID−19 infection: SI: 11.43, 7.18, 3.09, 8.04, 6.25;** **PEG 2000:** **$n = 2$ patients: SI: 4.57, 3.1** Negative: BNT-vaccine: $n = 2$ patients PEG 2000: $n = 4$ patients All vaccinated and not vaccinated controls ($n = 8$) and 5 controls after COVID−19 infection: Negative to all substances	11 out of 17 patients tolerated second dose of BNT-vaccine after a negative allergological work-up	Wortmannin experiments confirmed that positive basophil activation was mediated by IgE.	Labella M et al. 2021, September[28]

TABLE 2 (Continued)

Test (activation marker)	Tested substances	Concentration(s)/dilution(s)	Patients/controls for cellular tests	Threshold	Results	Patient(s) (re) vaccinated	Comments	Literature (order according to time of publication)
BAT^{Detail} (CD63)	S-vaccine Spike peptides	S-vaccine: 2 dilutions: 1:10, 1:100 Spike peptides: 2 dilutions: 1:100 and 1:1000	S-vaccine suspected allergic patients: $n = 7$ No controls	Stimulation index ≥2 and activated basophils >5%	S-vaccine: Negative: $n = 7$ patients Spike peptides: Negative: $n = 7$ patients	All seven patients tolerated revaccination.	Spike peptides: Average percentage slightly higher than baseline (2.16% vs. 0.65%)	Triwatcharikorn et al. 2021, October[30]
BAT^{Detail} (CD63)	PEG 2000 PEG-Dox	Not published	BNT-vaccine suspected allergic patients: $n = 3$	>5% activation	Negative: $n = 2$, 1 non-responder	2nd vaccination: $n = 1$: Small wheal after 1 h $n = 2$: Negative within 1h		Duque et al. 2021, November[10]
BAT^{Detail} (CD63)	BNT-vaccine M-vaccine AZ-vaccine J-vaccine PEG 2000 DMG-PEG 2000 PEG 3350 PEG 4000 PEG 6000 Poly 80	Vaccines: 4 conc. (1:125 dilution – undiluted: 4.4 corresponding to 0.18/0.36 μg/ml to 22.7/45.45 μg/ml for the BNT/M-vaccine), PEG 2000: 0.036 mg/ml–15 mg/ml DMG-PEG 2000: 3 conc.: 0.0000728 mg/ml–0.00182 mg/ml, PEG 3350:3 conc.: 0.6 mg/ml–15 mg/ml PEG 4000: 6 conc. 0.036 mg/ml–15 mg/ml PEG 6000: 3 conc.: 0.6 mg/ml–15 mg/ml Poly 80: 2 conc. 2.3 mg/ml–22.7 mg/ml	PEG patients: $n = 4$, Vaccinated controls: $n = 3$	≥15% activation	**Patients—Positive:** **BNT-vaccine: 3 patients: max. activation: 34.4%, 37.2%, 21.3%** **M-vaccine: $n = 4$ patients: max. activation: 34.8%, 16.1%, 41.8%, 20.5%** **PEG 4000: $n = 1$ patient: 16.2% activation** **PEG 6000 $n = 1$ patient: 35.8% activation** Negative: AZ-vaccine, J-vaccine, PEG 2000, DMG-PEG 2000, PEG 3350, Poly 80: $n = 4$ patients 3 controls: Negative to all substances	Vaccination with AZ-vaccine tolerated: $n = 3$ Not vaccinated yet: $n = 1$	Systemic reactions after SPT: 3 patients Convincing history of PEG allergy: 1 patient	Brockow K et al. 2021, November[26]
BAT^{Detail} (CD63)	BNT-vaccine DMG-PEG 2000	BNT-Vaccine: 1 μl DMG-PEG 2000: 0.002 μg/μl	BNT-vaccine suspected reactive patient: $n = 1$ No control	Not published	**Patient—Positive:** **BNT-vaccine: 23.3% activation** **DMG-PEG 2000: 29.1% activation**	No second vaccination so far		Jiang et al. 2021, December[9]

Note: *AZ-vaccine*: COVID-19 vaccine AstraZeneca, Vaxzevria®, AstraZeneca; *BNT-vaccine*: BNT162b2, Comirnaty®, Pfizer-BioNTech; *J-vaccine*: Janssen COVID-19 Vaccine, Johnson and Johnson; *M-vaccine*: COVID-19 vaccine Moderna, Spikevax®, Moderna.

Abbreviations: ALC-0159 PEG 2000, 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ingredient of BNT-vaccine); BAT, basophil activation test; BHRT, basophil histamine release test; conc., concentration; Detail, detailed description of method or commercially available test; DMG-PEG, polyethylene glycol 2000 dimyristoyl glycerol (ingredient of M-vaccine); HR, histamine release; max., maximal; n.a., not applicable; PEG, polyethylene glycol; PEG-Dox, PEGylated liposomal doxorubicin; Poly, polysorbate; Ref, reference for method mentioned; SPT, skin prick test.

Bold print indicate positive results.

7 | EVALUATION OF THE STUDIES

7.1 | General aspects

This review after the first year of COVID-19 vaccination shows that data on the value of cellular tests in the prevention and diagnosis of allergic reactions to COVID-19 vaccines are increasing, but results are limited. This may be due to the rare numbers of true allergic reactions to COVID-19 vaccines, as well as the low number of PEG-allergic patients with PEG being the component of the COVID-19 vaccine supposed to be the trigger of such reactions. However, the number of centres performing cellular tests and the availability of vaccines for *in vitro* experiments are limited. With regard to the validation of the BAT, the studies performed in 2021 have mainly been carried out with established or certified methods, so that the basis of the data appears reliable. PEG patients had a convincing history, while the other patients were vaccine suspected reactive patients. The performance of a provocation test, the gold standard of allergy diagnostics, has been reported in only few cases.

7.2 | mRNA vaccines vs. other PEG components, cross-sensitization to polysorbate 80

Nevertheless, it can be observed that in PEG-allergic patients, cellular tests with mRNA-vaccine showed clear positive results. The BNT-vaccine contains 50 μg/dose PEG lipids.[5] The best concentration of the whole BNT-vaccine giving positive results in the BAT was around 10 μg/ml tozinameran.[8,26,27,28] Dose-response curves should include this concentration, though lower and higher concentrations can be successfully used.

Basophil activation with these mRNA vaccines was higher and, in more cases, positive compared to other PEG components. Of these, the PEG 2000 derivatives, DMG-PEG 2000 or PEGylated doxorubicin 2000–3500, seemed to be preferable alternatives for confirming a PEG allergy or PEG-based allergy to mRNA vaccines. Considering newer as well as previous data prior to 2020, it is suggested to avoid unmodified PEGs with MW <2000 as they were not successful (except for one case with PEG 1500). Unmodified PEGs with a higher MW (2000–20,000) were only occasionally positive.

Polysorbate 80 was negative in recent cellular tests in PEG-allergic patients (Table 2). Therefore, cross-sensitization does not seem to be relevant.

7.3 | Mechanisms

Positive results with mRNA vaccines in cellular tests seem to indicate a PEG allergy, but confirmation with provocation tests was only performed in a minority of studies. It was assumed that the PEG conformation on the surface of nanoparticles results in an increased avidity augmenting IgE-crosslinking on the surface of basophils.[27] Furthermore, it was postulated that only repetitive presentation of the structure in the form of a polymer chain induces a biological response.[13] Due to previous and actual experiments in this context, an IgE-mediated mechanism for PEG allergy can be assumed. In a PEG-allergic patient, Wenande et al. (2013) showed that passive sensitization of IgE-stripped donor basophils with patient serum, and subsequent challenge with PEG 6000 and

TABLE 3 Summary of substances most often positive in cellular tests in patients with suspected allergy to PEG or COVID-19 vaccines (for details, see Table 2)

Substances	Number of positive results (positive/total)	Results
BNT-vaccine	3/3 PEG patients	51%–82.1% $CD63^+$ basophils[27]
	1/9 vaccine suspected reactive patients	1 positive BHRT[29]
	4/6 vaccine suspected reactive patients	2.88–4.79 SI in the BAT[28]
	3/4 PEG patients	21.3%–37.2% $CD63^+$ basophils[26]
	1/1 vaccine suspected reactive patient	23.3% $CD63^+$ basophils[9]
M-vaccine	1/9 vaccine suspected reactive patients	1 positive BHRT[29]
	4/4 PEG patients	16.1%–41.8% $CD63^+$ basophils[26]
mRNA vaccines (not specified)	13/13 vaccine suspected reactive patients	13%–74% $CD63^+$ basophils[8]
DMG-PEG	12/13 vaccine suspected reactive patients	10%–73% $CD63^+$ basophils[8]
	0/9 vaccine suspected reactive patients	Negative BHRT[29]
	0/4 PEG patients	Negative BAT[26]
	1/1 vaccine suspected reactive patient	29.1% $CD63^+$ basophils[25]
PEG-Dox	3/3 PEG patients	22.3%–35.4% $CD63^+$ basophils[27]
	0/3 vaccine suspected patients	Negative BAT, 1 Non-responder[10]

Note: BNT-vaccine: BNT162b2, Comirnaty®, Pfizer-BioNTech; M-vaccine: COVID-19 vaccine Moderna, Spikevax®, Moderna.

Abbreviations: BAT, basophil activation test; BHRT, basophil histamine release test; DMG-PEG, polyethylene glycol 2000 dimyristoyl glycerol (ingredient of M-vaccine); PEG, polyethylene glycol; PEG-Dox, PEGylated liposomal doxorubicin.

the culprit drug-containing PEG 3350 showed positive histamine release. Patient serum incubated with omalizumab (IgE-blocking antibodies) prior to passive histamine release tests abolished PEG-mediated histamine release.[13] In a basophil histamine release inhibition study, PEG 3350 and PEG 6000-induced histamine release were abolished by preincubation with a monomer or dimer. This inhibition appeared to be antigen-specific, as anti-IgE-induced histamine release remained unchanged after preincubation with the monomer as well as dimer. These results strongly indicate that serum factors in the patient's blood, possibly IgE antibodies, may bind monovalent ethylene glycol.[13] Similar results were found in mice sensitized to PEGylated asparaginase (PEG-MW = 5 kDa) and pre-treated with PEG 400 Da.[32] Wortmannin experiments also confirmed that basophil activation by PEG or BNT-vaccine was mediated by IgE.[28] Some authors have claimed that a non-IgE-mediated mechanism is the cause of basophil activation, because IgE against PEG could not be found in the serum.[10,28] The lack of detection could be due to methodological problems in these assays, because a new dual cytometric bead assay (using PEGylated products) was able to demonstrate that samples of patients with PEG-associated anaphylaxis were clearly positive for anti-PEG-IgE.[33] Direct basophil activation by the vaccines as a mechanism can possibly be excluded because of the vast majority of negative results in controls (Table 2).

7.4 | Problems of interpretation

The definition of a threshold for positivity in the BAT was very different in the various studies, ranging from >4% to >15% with different 'other' conditions (>25% of the positive controls or use of a stimulation index). ROC curves were performed in one study with PEG 2000 and BNT-vaccine but were based on a very low number of positive results.[28]

Even if in most studies with these vaccines, vaccinated and unvaccinated controls were negative in the cellular tests, one problem was the positive results observed in half of the controls due to a previous SARS-CoV-2 infection (vaccinated and non-vaccinated) in one study.[28] In the BHRT, a marginally positive result was also found in a control with a previous SARS-CoV-2 infection.[29] It is known that the SARS-CoV-2 infection induces complement activation, which could activate basophils. Furthermore, anti-SARS-CoV-2-specific IgE and mast cells with positive staining for IgE and CD63 were observed in patients with severe SARS-CoV-2 infections.[34] Experiments with the BAT showing a decrease in BNT-vaccine-induced basophil activation by preincubation with wortmannin might pose an argument for an IgE-mediated immune response to SARS-CoV-2 spike proteins in patients with a previous SARS-CoV-2 infection.[28] BATs with spike peptides showed a slightly higher activation compared to baseline (below the cut-off) in some patients with previous SARS-CoV-2 infection, which could be a hint for such a mechanism.[30] On the other hand, PEGylated compounds have the role of a solubilizer during the transition of the particles into the intracellular cytosol due to their hygroscopic properties,[35] which might influence unspecific detection or upregulation of surface markers on basophils in certain individuals.

This problem and the various thresholds used in the studies make it difficult to define a clear upper cut-off for a positive result with mRNA vaccines, but values of <5% basophil activation for vaccines and PEGs have been uniformly interpreted as a clear negative result throughout all studies. The tolerance of a COVID-vaccine negative in BATs and BHRTs was shown in a series of cases.[9,26,28] These results seem to reflect a good negative predictive value of these cellular tests, although more data on the tolerance of PEG-containing drugs or vaccines in such cases are necessary.

8 | CONCLUSION AND RECOMMENDATIONS

Cellular tests (preferably BAT) can successfully be integrated into the allergy test procedure, if there is a convincing history of an immediate-type reaction to a COVID-vaccine or PEGs. As skin testing in PEG-allergic patients can induce systemic anaphylactic reactions,[26,36] cellular tests could be performed before. Preference should be given to mRNA vaccines or modified compounds containing PEG over unmodified PEGs, which less often lead to positive results. Positive results (threshold in the BAT to be defined, probably >10%–15% activated basophils) seem to indicate a PEG allergy. A series of concentrations should be used to obtain the dose-response curves. Previous COVID infections should be considered if the results for mRNA vaccines are unexpectedly positive. A negative BAT (<5% activated basophils) or BHRT to a vaccine should encourage vaccination with the tested vaccine. (Table 4).

TABLE 4 Main conclusions regarding cellular tests in the context of allergy to COVID-19 vaccines

Prerequisites	Experience with cellular tests preferably BAT (internal test controls, tests with individuals tolerating the drugs, performance of dose-response-curves with different concentrations)
	Availability of vaccine remnants Modified compounds containing PEG can additionally be used.
Patient selection	Convincing history of immediate-type reaction to a COVID-19 vaccine or PEGs
Interpretation	A negative BAT (<5% activated basophils) to a vaccine (together with a negative skin test) should encourage vaccination with the tested vaccine
	A positive BAT (threshold to be defined, possibly >10%–15% activated basophils) or BHRT to a vaccine should result in vaccine administration with an alternative vaccine tested negative or with the positive tested vaccine under close observation and emergency preparedness

9 | UNMET NEEDS AND OUTLOOK

To calculate the sensitivity, specificity, and negative and positive predictive values of the cellular tests with COVID-19 vaccines and define the exact thresholds for a positive result, these *in vitro* tests must be performed in a larger number of patients suspected of a definite immediate-type allergic reaction to COVID-19 vaccines or PEGs, after a careful allergological work-up including provocation tests. Due to the small number of patients, this should be performed in a multicentre setting.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

B.E. has made substantial contributions to the conception of this manuscript. S.M., J.F., U.D., T.B., and K.B. have made contributions in this review.

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