

Use of basophil activation test in the investigation of adverse effects to vaccines

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Diagnosis of adverse effects caused by vaccines mainly includes investigative approaches based on skin tests, histamine, tryptase and serum IgE levels which should allow to diagnose an IgE-mediated allergy reaction, most caused by additive components. Incidence of vaccine-related allergy and anaphylaxis due to vaccination are very poorly represented among population, based on the latest epidemiological and safety reports. Notwithstanding, routinely allergy diagnostic tools show many pitfalls and contradictory results when used to highlight an allergy onset or a clinical manifestation recalling an hypersensitivity reaction. Furthermore, adjuvants in vaccines may trigger innate cells response by toll-like receptors, thus eliciting a possible non-IgE mediated allergy phenomenon or causing B-cell activation and autoimmunity. A cellular test, such as a basophil activation test, or more widely a flow cytometry analysis of immune cells, might add new insights to the comprehension of allergy due to vaccination, thus ameliorating the diagnostic endowment in the hand of physicians.

Hypersensitivity to vaccination represents one of the main concerns raised about possible reactions to vaccines; however, according to recently reported data the rate of allergic reactions due to vaccines is quite low but, at the same time, a true relationship between routine vaccines and allergy onset has not yet been outlined, leading to the consideration that the number of true allergic reactions primarily caused by vaccination is not known.¹ In those subjects with an immune system

characterized by high levels of immaturity or senescence, namely children and elderly people, the risk for adverse reactions to vaccines should elicit main concerns by physicians. The fact is that as new vaccines are actually added to the immunization schedule provided for children, to be administered concurrently with other vaccines, it is predictable that acute allergic reactions might increase.² Notwithstanding, the development of vaccines has been one of the most important achievement in preventive medicine; as the incidence of vaccine-preventable diseases is reduced by immunization, general public becomes increasingly concerned about the safety associated with vaccination. Vaccine safety is extensively evaluated through animal safety studies, clinical trials, during manufacturing processes and post-licensure surveillance. Safety monitoring in post-licensure surveillance has relied on passive reporting system and epidemiological studies. In Italy, some good experience to prevent noxious side-effects due to routine vaccines, has been performed.^{3,4} The latest survey accomplished by the Italian colleagues, made with the purpose to update the activity led by Green Channel in a period spanning about 15 y, has shown that allergic reactions to vaccines are very few⁴ and that even anaphylactic reactions should be reappraised and deeply investigated, when clinical and laboratory outputs would suggest their occurrence.^{5,6} Anyway, medical literature, is affected by episodes, though sporadic, that cast very serious concerns in ordinary people about vaccine safety.⁷⁻⁹ Despite this, a forthright relation between vaccinations and the

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reported episodes has shown as uncertain. Safety checking about allergic reactions is mainly centered on minor protein components included in adjuvanted vaccines that possibly trigger an anaphylactic response.¹⁰ Presumptive allergy following a vaccination episode often accounts on diagnostic tests that might not solve the diagnostic suspect; in addition a delayed-hypersensitivity reaction may occur. So, besides to inform physicians and pediatricians as well as parents about possible severe complications after the administration of vaccines, extensive studies are requested in order to assess or exclude a relation between vaccination and serious impairments, mainly in infants.⁷ These studies are however hampered by many hindered issues relating host immune response to vaccine, which reflects the complex scenario of host-pathogen interaction.¹¹ Furthermore, this picture is rather worsened by misleading health-related information found on the web, which is currently increasing and can affect patient decision making, e.g., regarding vaccination, even when good and reliable statistical information is presented concurrently, so forcing physicians to upgrade their efforts in this field.¹² As regards allergy to vaccine, is certainly very hard to plan a perspective aiming at screening any individual who is undergoing to a forthcoming vaccination: in vitro allergy test during preparation and the very low frequency of acute allergy onset due to vaccines should ensure about their safety.^{7,8} Allergy diagnosis related to an adverse vaccination yet accounts on anamnesis, skin tests and serum IgEs level. The use of cellular assays, such as basophil activation test (BAT) is rarely considered. BATs are flow cytometry assays which evaluates the activation response of basophil toward an allergen or an agonist by measuring the upregulation of membrane molecules, such as CD63 or CD203c, through a flow cytometry evaluation. To catch some contributing suggestion about the suitability in using a BAT in vaccine adverse effect diagnosis, maybe we should consider the usefulness of BAT in other fields of investigation, as very little is known about their feasibility in allergy diagnosis to vaccines. A suggestion might come, for example, from the application of BAT in allergen

immunotherapy,¹³ as immunotherapy can be considered a vaccination, for certain aspects. Basal mechanisms underlying the mode of action of allergen-specific immunotherapy include many steps comparable to the immune response to vaccine: a modulation of T-and B-cell responses and related antibody isotypes, the migration of inflammatory cells to tissues and the involvement of Th1/Th2 balance and T-regulatory cells as key regulators of peripheral tolerance to allergens¹⁶ and allergen immunotherapy, as vaccination, certainly focuses onto the role of innate cells in the therapeutic outcome. This wide stage of immune events directly involve basophil, just in the first line of intervention.¹⁷ Modulation of basophil response to allergen immunotherapy is a complex mechanism that involves primarily innate cells and claims for an upgrading in the consideration of cellular test for allergy diagnosis.¹⁸ Vaccines induce innate immunity to work;¹⁹ a role for this involvement is ruled by vaccine adjuvants, most of which target Toll-like receptors (TLRs) in innate cells, such as basophils.¹⁹⁻²¹ TLRs are expressed also on T-cell membranes and participates in the regulation of both T and T-regulatory cells in the immune system,²² so contributing also to vaccination success. Although a strategy to develop vaccines deprived from TLRs has been proposed recently,²³ the activation of TLRs, either directly or through other pattern recognition receptors and signaling machinery, may activate basophils and induce a non IgE-mediated allergic response.²⁴ A recently published report assessed the role of TLRs in newborns from atopic or asthmatic mothers and showed that TLR2-mediated innate immune responses play a role in increasing the incidence of allergic reactions through the modulation of basophil cytokine secretion and histamine release; furthermore, microbial components released by bacteria, such as formylated peptides, during life, may activate basophils through TLR2 (especially for genetically predisposed infants) to release cytokines associated with an increased incidence of allergic diseases.²⁵ Activation of non IgE-mediated pathways should elicit CD63 and CD203c upregulation in basophils while skin tests might result negative.²⁶ This appear to

represent also a critical issue for the explanation of other chronic ailments involving TLRs in vaccine adjuvants.^{27,28} BAT appears then potentially feasible for allergy to vaccines and generally for adverse reaction to adjuvants. The use of BAT to reach insights about vaccine hypersensitivity could be associated to other routinely used allergy tests. BAT choice would enrich the diagnostic potential of cellular tests by flow cytometry in order to deepen allergic mechanisms by in vitro evaluation, so upgrading the ability of physicians to highlight an allergic event. Immunology of allergy is gaining more and more cues about its complex picture. In this context many other issues should be reappraised in next future. For example, histamine and tryptase levels might not be sufficient to assess an anaphylactic process, as basophils and even neutrophils can trigger an anaphylactic reaction in a non-IgE, non-histamine mediated process and because tryptase levels may not correlate with anaphylaxis.^{29,30} Basophils are triggered by many agents that do not fulfil the classical scheme of IgE-mediated reactions. As a matter of fact, suspected allergic reactions to biological substances are reported in about 10% of children and one has to distinguish a real "allergy onset" from a "pseudo-allergy."³¹ Non-immediate reactions may also result from complex interactions between vaccine components, immune system and "danger signals" provided or induced by infectious and/or inflammatory diseases. Diagnosis is based above all on a detailed analysis of clinical history, skin tests (if validated) and challenge tests (if indicated). At present, the diagnostic and predictive values of in vitro tests exploring immediate (specific IgE determination, histamine and leukotriene release tests, basophil activation test) and non-immediate type (lymphocyte activation tests and cytokine assays in the supernatant of activated blood mononuclear cells) of vaccine adverse effects are not yet validated but might address research on vaccine side-effects and improve both diagnostic approaches and our understanding of immune response to vaccination. A cellular test, such as a basophil activation test, or more widely a flow cytometry analysis of immune cells, might add new insights to the comprehension of

allergy due to vaccination, thus ameliorating the diagnostic endowment in the hand of physicians.

References

- Fritsche PJ, Heilbling A, Ballmer-Weber BK. Vaccine hypersensitivity-an overview. *Swiss Med Wkly* 2010; 140:238-46; PMID: 20349363.
- Thibodeau JL. Office management of childhood vaccine-related anaphylaxis *Can Fam Physic* 1994; 40:1602-10.
- Zanoni G, Ferro A, Valsecchi M, Tridente G. The "Green Channel" of the Veneto region as a model for vaccine safety monitoring in Italy. *Vaccine* 2005; 23:2354-8; PMID: 15755627; DOI: 10.1016/j.vaccine.2005.01.021.
- Micheletti F, Moretti U, Tridente G, Zanoni G. Consultancy and surveillance of post-immunization adverse events in the Veneto region of Italy for 1992–2008. *Hum Vaccin* 2011; In press; PMID: 21301223; DOI: 10.4161/hv.7.0.14613.
- Corvo M, Martelli A, Gemellaro L, Fiocchi A. Recognizing anaphylaxis in the emergency department. *Pediatr Med Chir* 2008; 30:16-24; PMID: 18491674.
- Zanoni G, Gottin L, Boner A, Piacentini G, Peroni D, Gold M. Case discussion of an immediate serious reaction to hexavalent vaccine mistaken for anaphylaxis. *Br J Clin Pharmacol* 2010; 70:916-7; PMID: 21175450; DOI: 10.1111/j.1365-2125.2010.03771.x.
- Zinka B, Rauch E, Buettner A, Ruëff F, Penning R. Unexplained cases of sudden infant death shortly after hexavalent vaccination. *Vaccine* 2006; 24:5779-80; PMID: 15908063; DOI: 10.1016/j.vaccine.2005.03.047.
- von Kries R. Comment on Zinka B, et al. Unexplained cases of sudden infant death shortly after hexavalent vaccination. *Vaccine* 2006; 24:5783-4; PMID: 16081190; DOI: 10.1016/j.vaccine.2005.03.055.
- Schmitt HJ, Siegrist CA, Salmaso S, Law B, Booy Zinka RB, et al. Unexplained cases of sudden infant death shortly after hexavalent vaccination. *Vaccine* 2006; 24:5781-2; PMID: 16084630; DOI: 10.1016/j.vaccine.2005.03.054.
- Gagnon R, Primeau MN, Des Roches A, Lemire C, Kagan R, Carr S, et al. PHAC-CIHR Influenza Research Network. Safe vaccination of patients with egg allergy with an adjuvanted pandemic H1N1 vaccine. *J Allergy Clin Immunol* 2010; 126:317-23; PMID: 20579720; DOI: 10.1016/j.jaci.2010.05.037.
- Aderem A, Adkins JN, Ansong C, Galagan J, Kaiser S, Korth MJ, et al. A systems biology approach to infectious disease research: innovating the pathogen-host research paradigm. *MBio* 2011; 2:e00325-10; DOI: 10.1128/mBio.00325-10; PMID: 21285433.
- Betsch C, Ulshöfer C, Renkewitz F, Betsch T. The Influence of Narrative v. Statistical Information on Perceiving Vaccination Risks. *Med Decis Making* 2011; In press; PMID: 21447730; DOI: 10.1177/0272989X11400419.
- Dhillon S. Spotlight on DTPa-HBV-IPV/Hib Vaccine (Infanrix hexa). *BioDrugs* 2010; 24:299-302; DOI: 10.2165/11206690-000000000-00000; PMID: 20795752.
- Moraga Llop FA, Salleras Sanmartí L. [Safety of hexavalent combined vaccines and the principle of precaution]. *An Pediatr (Barc)* 2006; 65:573-7; PMID: 17194328; DOI: 10.1157/13095851.
- Kucera P, Cvackova M, Hulikova K, Juzova O, Pachel J. Basophil activation can predict clinical sensitivity in patients after venom immunotherapy. *J Invest Allergol Clin Immunol* 2010; 20:110-6; PMID: 20461965.
- Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2011; 127:18-27; PMID: 21211639; DOI: 10.1016/j.jaci.2010.11.030.
- Schneider E, Thieblemont N, De Moraes ML, Dy M. Basophils: new players in the cytokine network. *Eur Cytokine Netw* 2010; 21:142-53; PMID: 20837449.
- Chirumbolo S, Zanoni G, Ortolani R, Vella A. In vitro Biphasic Effect of Honey Bee Venom on Basophils from Screened Healthy Blood Donors. *Allergy Asthma Immunol Res* 2011; 3:58-61; PMID: 21217927; DOI: 10.4168/air.2011.3.1.58.
- Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. *Immunity* 2010; 33:492-503; PMID: 21029960; DOI: 10.1016/j.immuni.2010.10.002.
- Marćiani DJ. Vaccine adjuvants: role and mechanisms of action in vaccine immunogenicity. *Drug Discov Today* 2003; 8:934-43; PMID: 14554157; DOI: 10.1016/S1359-6446(03)02864-2.
- Jiang ZH, Koganty RR. Synthetic vaccines: the role of adjuvants in immune targeting. *Curr Med Chem* 2003; 10:1423-39; PMID: 12871139; DOI: 10.2174/0929867033457340.
- Oberg HH, Juricke M, Kabelitz D, Wesch D. Regulation of T cell activation by TLR ligands. *Eur J Cell Biol* 2011.
- Ishii KJ, Akira S. Toll or toll-free adjuvant path toward the optimal vaccine development. *J Clin Immunol* 2007; 27:363-71; PMID: 17370119; DOI: 10.1007/s10875-007-9087-x.
- Yang IA, Fong KM, Holgate ST, Holloway JW. The role of Toll-like receptors and related receptors of the innate immune system in asthma. *Curr Opin Allergy Clin Immunol* 2006; 6:23-8; PMID: 16505608; DOI: 10.1097/01.all.0000200503.77295.bb.
- Yang L, Xu WG, Xu YP, Guo YS, Xiong Y, Shi GY. The Effect of Peptidoglycan Stimulation on Basophil-Mediated Atopic Responses during Pregnancy and in Newborns. *J Asthma* 2011; In press.
- McClimon B, Rank M, Li J. The predictive value of skin testing in the diagnosis of local anesthetic allergy. *Allergy Asthma Proc* 2011; 32:95-8; PMID: 21439161; DOI: 10.2500/aap.2011.32.3417.
- Booth J, Wilson H, Jimbo S, Mutwiri G. Modulation of B cell responses by Toll-like receptors. *Cell Tissue Res* 2011; 343:131-40; PMID: 20824286; DOI: 10.1007/s00441-010-1031-3.
- Israeli E, Agmon-Levin N, Blank M, Shoenfeld Y. Adjuvants and autoimmunity. *Lupus* 2009; 18:1217-25; PMID: 19880572; DOI: 10.1177/0961203309345724.
- Jönsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al. Mouse and human neutrophils induce anaphylaxis. *J Clin Invest* 2011; 121:1484-9; DOI: 10.1172/JCI45232; PMID: 21436586.
- Sargur R, Cowley D, Murng S, Wild G, Green K, Shrimpton A, et al. Raised tryptase without anaphylaxis or mastocytosis: heterophilic antibody interference in the serum tryptase assay. *Clin Exp Immunol* 2011; 163:339-45; PMID: 21303361; DOI: 10.1111/j.1365-2249.2010.04287.x.
- Ponvert C, Scheinmann P. Vaccine allergy and pseudo-allergy. *Eur J Dermatol* 2003; 13:10-5; PMID: 12609774.