

Hen Egg White Lysozyme Vaccination Induces Acute Shock in NOD Mice

DUMITRU D. BRANISTEANU,^a LUTGART OVERBERGH,^b
BRIGITTE DECALLONNE,^b AND CHANTAL MATHIEU^b

^a*Department of Endocrinology, University of Medicine and Pharmacy “Gr. T. Popa” Iasi, Iasi, Romania*

^b*Laboratorium voor Experimentele Geneeskunde en Endocrinologie (LEGENDO), Katholieke Universiteit Leuven, Leuven, Belgium*

ABSTRACT: To investigate whether vaccination could induce lethal shock and which mechanisms are involved in this phenomenon, we tested a panel of autoantigens or diabetes-irrelevant peptides or proteins in nonobese diabetic (NOD), Balb/c, and C57Bl/6 mice. Of the antigens tested, only nondiabetogenic hen egg white lysozyme induced a severe form of shock exclusively in NOD mice. The mechanism involved is suggestive of a Th₂-mediated anaphylactic reaction possibly connected to activation of PAF and triggering of DIC.

KEYWORDS: hen egg white lysozyme; NOD mice; shock; platelet-activating factor; diabetes; vitamin D

Several recent reports described the appearance of allergic or anaphylactic reactions subsequent to autoantigen vaccination in animal models of autoimmune diabetes mellitus¹ or multiple sclerosis,^{2–4} questioning the safety of this immune-modulating strategy in preventing the onset of T helper 1 (Th₁)–mediated diseases. To investigate whether vaccination could induce lethal shock and which mechanisms are involved in this phenomenon, we tested a panel of autoantigens or diabetes-irrelevant peptides or proteins in nonobese diabetic (NOD), Balb/c, and C57Bl/6 mice. Eight-week-old mice were immunized by injection of 100 µg antigen (hen egg white lysozyme [HEL], GAD65, insulin B, hsp65, proteolipid protein, ovalbumin, tetanus toxin), emulsified at a 1:1 concentration in complete Freund’s adjuvant (Difco Laboratories, Detroit, MI), in the hind foot pads and reinjected with the same antigen 3 weeks later.

Of all the antigens tested, only nondiabetogenic HEL induced a severe form of shock exclusively in NOD mice (TABLE 1). Administration of 1,25(OH)₂D₃ (a hormone known to favor a differentiation shift toward Th₂)⁵ at a dose of 5 µg/kg/2 days, starting from 3 weeks of age, further increased shock severity in NOD mice (increase

Address for correspondence: Chantal Mathieu, M.D., Ph.D., Gasthuisberg, LEGENDO, Onderwijs & Navorsing, Herestraat 49, 3000 Leuven, Belgium. Voice: +32-16-34-60-30; fax: +32-16-34-59-34.
chantal.mathieu@med.kuleuven.ac.be

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TABLE 1. Shock incidence and severity in various groups of mice rechallenged with HEL and pretreated ip with 1,25(OH)₂D₃ (+) or only with the vehicle (peanut oil, –)

Mouse strain	1,25-D ₃	Rescue therapy	Shock incidence (from total)	Shock severity	
				Lethal	Reversible
NOD	–	–	11/11	3 (27%)	8
	+	–	17/17	14 (82%)	3
	–	DEX	0/8	0	0
	+	DEX	0/8	0	0
	–	WEB2086	6/8	0	6
	+	WEB2086	6/8	0	6
Balb/c	–	–	0/9	0	0
	+	–	9/9	0	9
C57Bl/6	–	–	0/9	0	0
	+	–	0/9	0	0

NOTE: Certain groups of NOD mice were submitted to rescue therapy with dexamethasone (DEX) or WEB2086.

in lethality incidence from 27% to 82%, $p < 0.0001$) and led to the appearance of reversible shock signs also in Balb/c mice, while C57Bl/6 mice remained shock-resistant (TABLE 1).

The mRNA levels of IL-13 (Th₂-specific cytokine involved in anaphylaxis)⁶ measured by real-time PCR before HEL boost injection were significantly higher in spleen of NOD mice than in shock-resistant C57Bl/6 mice (38.1 ± 7.8 vs. 18 ± 2.2 IL-13 mRNA/actin $\times 10^{-6}$, $p < 0.05$). IL-13 was further increased by administration of 1,25(OH)₂D₃ in NOD mice (63.4 ± 6.9 IL-13 mRNA/actin $\times 10^{-6}$, $p < 0.05$), but not in C57Bl/6 mice (13 ± 4.9 IL-13 mRNA/actin $\times 10^{-6}$, ns). Serum total IgE levels (Th₂-dependent immunoglobulin involved in anaphylactic reactions),⁷ measured by ELISA before boost injection, were higher in NOD mice (mean OD of 690 ± 130 in NOD mice vs. 338 ± 47 in C57Bl/6 mice, $p < 0.05$). 1,25(OH)₂D₃ administration further increased IgE levels only in NOD mice (mean OD of 950 ± 104 , $p < 0.05$), but not in C57Bl/6 mice (mean OD of 376 ± 36 , ns). IgE hence displayed a parallel evolution with IL-13, suggesting a causal relationship, as previously described.⁷

Because the shock induced in NOD mice was suggestive of disseminated intravascular coagulation (DIC), we further investigated a possible role of platelet-activating factor (PAF) as a shock mediator. The expression of PAF receptor was higher in spleen of NOD mice than in C57Bl/6 mice ($96.3 \pm 15 \times 10^{-2}$ vs. $39.8 \pm 11 \times 10^{-2}$ PAF receptor mRNA/actin, $p < 0.05$), suggesting a hypersensitivity to PAF in HEL-sensitized NOD mice. PAF receptor expression in NOD mice was further increased by therapy with 1,25(OH)₂D₃ (from $96.3 \pm 15 \times 10^{-2}$ to $155 \pm 22 \times 10^{-2}$, $p < 0.05$), raising the possibility of a Th₂-dependent hypersensitivity to PAF.

The involvement of both immune effectors and PAF in the etiology of HEL-induced shock was further indicated by the efficacy of rescue therapy with dexamethasone, 50 μ g ip, 48, 24, and 2 h before the HEL booster injection, and

WEB2086 (PAF receptor antagonist, Boehringer Mannheim, Germany; kind gift from Prof. Vermeylen, University of Leuven, Leuven, Belgium), 500 µg ip, 10 min before booster injection, respectively (TABLE 1).

In conclusion, NOD mice are highly susceptible to lethal shock upon immunization with HEL. The mechanism involved is suggestive of a Th₂-mediated anaphylactic reaction possibly connected to activation of PAF⁸ and triggering of DIC. In the case of Th₁-mediated autoimmune diseases, balancing the immune system toward Th₂ differentiation might increase the risk of unwanted anaphylactic effects. Extreme caution should therefore be taken in using any kind of immune-modulating strategies of T helper differentiation in order to prevent excessive Th₂-dependent reactions.

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