

Depletion of Human Peripheral Blood Lymphocytes in CD25⁺ Cells Allows for the Sensitive *In Vitro* Screening of Contact Allergens

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TO THE EDITOR

Allergic contact dermatitis (ACD) is a frequent T-cell-mediated inflammatory skin disease in response to skin exposure to non-protein chemicals, called haptens. Development of ACD proceeds in two phases (Saint-Mezard *et al.*, 2004). The sensitization (afferent) phase, induced by the migration of hapten-loaded skin dendritic cells (DCs) to draining lymph nodes, leads to the priming of hapten-specific effector T cells. The elicitation (efferent) phase, generated on skin re-exposure to the same hapten, induces the recruitment of T cells into the skin resulting in ACD through IFN- γ production and cytotoxicity toward keratinocytes.

Allergic contact dermatitis is a major public health concern in industrialized countries, and there is an urgent need for tests allowing for detection of potentially allergenic compounds manufactured by industries (Diepgen and Kanerva, 2006; Williams *et al.*, 2006). Predictive testing for the sensitizing properties of haptens has been traditionally carried out in guinea pigs and mice (Gerberick *et al.*, 2007; Maurer, 2007). More recently, alternative *in vitro* methods have been developed to test the ability of haptens to prime naive peripheral blood T cells. These assays, referred to as primary sensitization assays, consist of an autologous mixed DC-lymphocyte reaction (MDLR) using hapten-loaded DCs as antigen-presenting cells and normal human peripheral blood leucocytes (PBLs) as responding cells. *In vitro* priming of hapten-specific T cells was usually assessed by cell proliferation

and/or T-cell cytokine production at the end of the 5- to 7-day culture (Krasteva *et al.*, 1996; Rustemeyer *et al.*, 1999; Rougier *et al.*, 2000). Although some strong experimental haptens were able to activate T cells in these primary sensitization assays, the magnitude of the T-cell response was routinely low and the results were poorly reproducible. One possible reason for the poor sensitivity of these assays may be the low frequency of hapten-specific T-cell precursors in normal individuals (Rougier *et al.*, 2000). Alternatively, *in vitro* priming of T cells may be repressed by naturally occurring CD4⁺CD25⁺ regulatory T cells (nTreg), which comprise up to 5% of the peripheral CD4⁺ T-cell pool and which are known to downregulate both the afferent and efferent phases of ACD (Vocanson *et al.*, 2006). In this respect, studies on contact hypersensitivity and oral tolerance to strong and weak haptens have shown that acute depletion of CD4⁺ T cells or CD25⁺ T cells in normal C57BL/6 mice results in a dramatic increase in the number of hapten-specific IFN- γ -producing T cells in lymph nodes draining the sensitization site (Dubois *et al.*, 2003; Vocanson *et al.*, 2006). Therefore, we postulated that *in vitro* T-cell priming to haptens might be more efficient if CD25⁺ cells were absent from the responding T-cell population. The data presented here confirm this hypothesis and show that depletion of PBLs in CD25⁺ cells increases the magnitude of hapten-specific T-cell sensitization *in vitro*.

A classical human autologous MDLR was used. DCs were generated from

6-day cultures of monocytes (isolated from peripheral blood of healthy donors) in complete RPMI medium supplemented with 10% fetal calf serum and recombinant human GM-CSF and IL-4 (R&D Systems, Lille, France). Monocyte-derived DCs were then incubated for 10 minutes with the strong/moderate haptens TNBS (2,4,6-trinitrobenzene sulfonic acid) and FITC, with the clinically relevant weak fragrance allergen HCA (α -hexylcinnamaldehyde) or with the non-sensitizers DMSO and sodium lauryl sulfate. After washing, haptenated DCs were cocultured for 5–7 days with either undepleted or CD25⁺-cell-depleted PBLs from normal human donors. Depletion of PBLs in CD25⁺ T cells was achieved using anti-CD25 mAb-coated microbeads and negative-selection columns (Mytenyi Biotech, Bergish Gladbach, Germany) and routinely resulted in 90% depletion of CD4⁺CD25⁺ T cells. Priming of hapten-specific T cells during the MDLR was determined by (i) analysis of cell proliferation following addition of ³[H]thymidine for the last 8 hours of culture and (ii) titration of IFN- γ production in culture supernatants after addition of a sub-mitogenic dose of phytohemagglutinin (PHA) for the last 15 hours of culture, as described previously (Vocanson *et al.*, 2006). Results were expressed as a stimulation index corresponding to the ratio of values recorded with hapten-pulsed versus unpulsed MDLC. Results show that depletion of naive PBLs in CD25⁺ T cells improves the sensitivity of the primary sensitization assay and allows for detecting the sensitizing properties of chemicals with moderate (FITC)/weak (HCA) allergenic properties. Indeed, classical primary autologous

Abbreviations: ACD, allergic contact dermatitis; DC, dendritic cell; MDLR, mixed DC-lymphocyte reaction; PBL, peripheral blood lymphocyte

Table 1. *In vitro* T-cell priming to contact sensitizers is improved in the absence of CD25+ cells

Chemical	Experiment no.	Cell proliferation						Cytokine secretion					
		Undepleted MDLR			CD25+ T-cell-depleted MDLR			Undepleted MDLR			CD25+ T-cell-depleted MDLR		
		³ [H]Thymidine incorporation (c.p.m. ± SD)			³ [H]Thymidine incorporation (c.p.m. ± SD)			IFN-γ secretion			IFN-γ secretion		
		Hapten-pulsed MDDC	Unpulsed MDDC	SI	Hapten-pulsed MDDC	Unpulsed MDDC	SI	Hapten-pulsed MDDC	Unpulsed MDDC	SI	Hapten-pulsed MDDC	Unpulsed MDDC	SI
TNBS (1,000 µg ml ⁻¹)	1	1,337 ± 1,707	2,561 ± 860	0.5	756 ± 369	90 ± 45	8.4	24	29	0.8	41	4	10.1
	2	1,163 ± 620	368 ± 11	3.2	2,192 ± 894	502 ± 216	4.4	6	6	1.0	37	7	5.3
	3	12,447 ± 2,204	4,760 ± 586	2.6	17,786 ± 3,066	1,385 ± 1,418	12.8	76 ± 2	50 ± 22	1.5	397 ± 62	89 ± 48	4.5
	4	3,137 ± 733	1,963 ± 337	1.6	6,424 ± 264	740 ± 529	8.7	43 ± 6	13 ± 4	3.3	72 ± 22	16 ± 6	4.5
	5	1,863 ± 755	706 ± 134	2.6	8,123 ± 390	338 ± 157	2.4	102 ± 8	89 ± 17	1.1	269 ± 51	73 ± 22	3.6
	6	8,643 ± 708	3,133 ± 406	2.8	6,168 ± 1,002	1,630 ± 307	3.8	41	31	1.3	197	32	6.2
	7	1,495 ± 611	1,245 ± 355	1.2	7,560 ± 2,136	315 ± 102	2.4	10 ± 3	9 ± 11	1	22 ± 11	7 ± 3	3
	8	6,769 ± 1,208	4,195 ± 649	1.6	6,070 ± 1,845	2,722 ± 723	2.2	15	16	0.9	225	11	20.5
	9	4,655 ± 1,242	2,253 ± 787	2.1	7,449 ± 1,178	2,068 ± 1,272	3.6	137	68	2	89	70	1.3
	10	1,141 ± 464	363 ± 78	3.1	9,798 ± 262	3,297 ± 1,246	3	29 ± 7	23 ± 17	1.3	519 ± 188	130 ± 4	4
	11	1,125 ± 115	1,086 ± 262	1	744 ± 452	432 ± 418	1.7	144	155	0.9	19	14	1.4
	12	ND	—	—	1,049 ± 802	102 ± 43	10.3	ND	—	—	137	44	3.1
	13	ND	—	—	5,653 ± 1,381	1,193 ± 547	4.7	ND	—	—	222	20	11.1
FITC (300 µg ml ⁻¹)	5	650 ± 218	706 ± 134	0.9	1,036 ± 488	338 ± 157	3.7	87 ± 33	117 ± 47	0.7	222 ± 29	202	1.1
	6	2,930 ± 883	3,133 ± 406	0.9	2,836 ± 350	1,630 ± 307	1.7	16	31	0.5	52	32	1.6
	8	5,859 ± 2,035	4,195 ± 649	1.4	6,800 ± 2,034	2,722 ± 63	2.5	9	16	0.6	41	11	3.7
HCA (130–400 µg ml ⁻¹)	1	663 ± 228	2,561 ± 860	0.25	499 ± 71	90 ± 45	5.5	ND	—	—	ND	—	—
	2	407 ± 152	368 ± 11	1.1	570 ± 227	502 ± 216	1.1	5	6	0.8	3	7	0.4
	7	1,871 ± 615	1,245 ± 355	1.5	275 ± 71	315 ± 102	0.9	10 ± 1	9 ± 11	1	7	7 ± 3	1
	9	2,335 ± 514	2,253 ± 787	1	4,202 ± 1,181	2,068 ± 1,272	2	88	68	1.3	153	70	2.2
	10	766 ± 64	364 ± 78	2.1	11,237 ± 3,951	3,287 ± 1,246	3.4	25 ± 20	23 ± 17	1.1	218 ± 91	130 ± 4	1.7
	11	1,219 ± 349	1,086 ± 263	1.1	851 ± 626	432 ± 418	2	136	155	0.9	12	14	0.8
	12	ND	—	—	156 ± 43	102 ± 43	1.5	ND	—	—	31	44	0.7
DMSO (1,000 µg ml ⁻¹)	5	666 ± 149	706 ± 134	0.9	248 ± 57	338 ± 157	0.7	100 ± 21	119 ± 47	0.8	203 ± 68	202	1
	6	3,052 ± 383	3,133 ± 406	1	1,733 ± 485	1,630 ± 307	1.1	10	31	0.3	22	32	0.7
	8	5,547 ± 2,381	4,195 ± 649	1.3	2,650 ± 347	2,722 ± 63	1	14	16	0.9	5	11	0.5
SLS (4–40 µg ml ⁻¹)	1	1,091 ± 125	2,561 ± 860	0.4	172 ± 108	90 ± 45	1.9	ND	—	—	ND	—	—
	2	366 ± 201	368 ± 11	1	939 ± 230	502 ± 216	1.9	ND	—	—	ND	—	—
	12	ND	—	—	125 ± 34	102 ± 43	1.2	ND	—	—	17	44	0.4

HCA, α-hexylcinnamaldehyde; MDDC, monocyte-derived dendritic cells; MDLR, mixed dendritic cell-lymphocyte reaction; ND, not determined; PHA, phytohemagglutinin; SI, stimulation index; SLS, sodium lauryl sulfate; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

A total of 4–8 × 10⁵ hapten-pulsed and unpulsed MDDCs (1,000 µg ml⁻¹ TNBS, 300 µg ml⁻¹ FITC, 130–400 µg ml⁻¹ HCA, 1,000 µg ml⁻¹ DMSO, or 4–40 µg ml⁻¹ SLS) were cultured for 5–7 days with 2 × 10⁵ autologous lymphocytes (depleted or not depleted in CD25+ cells). The proliferative responses were assayed by ³[H]thymidine incorporation (1 µCi per well) for the last 8 hours of culture. INF-γ secretion was measured by ELISA in the supernatant of the cultures restimulated for the last 15 hours with 2 µg ml⁻¹ PHA. Lymphoproliferation and INF-γ secretion induced by hapten-pulsed MDDC were compared with those that were obtained with unpulsed MDDC, and results are expressed as SI: response recorded for hapten-pulsed MDDC/response recorded for unpulsed MDDC. Results are considered positive for SI > 2 (bold values).

MDLR, that is, using undepleted PBLs as responding cells, resulted in (i) a moderate proliferative response to TNBS (in 6/11 experiments) with IFN- γ production in only 2/11 experiments; (ii) no proliferative response and IFN- γ production to FITC; and (iii) proliferative response to HCA in only 1/6 experiments without IFN- γ production. In contrast, depletion of naive PBLs in CD25 + T cells increased the sensitivity of the MDLR inasmuch as (i) a strong T-cell proliferation and IFN- γ production in response to TNBS was observed in almost all (12/13) experiments and (ii) mild but significant proliferative responses were obtained in 2/3 experiments (with IFN- γ production in 1/3 experiments) for FITC and 4/8 experiments (with IFN- γ production in 1/7 experiments) for HCA; (iv) the non-sensitizers, DMSO and sodium lauryl sulfate, did not induce any PBL proliferation or IFN- γ production. Therefore, depletion of PBLs in CD25 + T cells allows for optimal *in vitro* priming of T cells against contact sensitizers but not against xenobiotics devoid of sensitizing properties (DMSO, sodium lauryl sulfate).

In conclusion, this study confirms the hypothesis that regulatory cells may prevent the priming/expansion of specific T-cell precursors and further

suggests that *in vitro* primary sensitization assays using CD25 + -cell-depleted PBLs could be sensitive methods to test for the potential immunogenic properties of haptens, especially those endowed with weak sensitizing properties, such as fragrances and dyes (Vocanson *et al.*, 2006) (Table 1).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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