

Chapter 7

Human T Cell Priming Assay: Depletion of Peripheral Blood Lymphocytes in CD25⁺ Cells Improves the In Vitro Detection of Weak Allergen-Specific T Cells

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Abstract To develop an in vitro assay that recapitulates the key event of allergic contact dermatitis (ACD), that is the priming of effector T cells by hapten-presenting dendritic cells, and then allows for the sensitive detection of chemical allergens represents a major challenge. Classical human T cell priming assays (hTCPA) that have been developed in the past, using hapten-loaded monocyte-derived dendritic cells (MDDCs) as antigen-presenting cells and peripheral blood lymphocytes (PBLs) as responding cells, were not efficient to prime T cells to common allergens with moderate/weak sensitizing properties. Recent progress in the understanding of the effector and regulatory mechanisms of ACD have shown that T cell priming requires efficient uptake of allergens by immunogenic DCs and that it is controlled by several subsets of regulatory cells including CD25⁺ Tregs. We therefore analyzed various parameters involved in allergen-specific T cell activation in vitro and showed that priming of allergen-specific T cells is hampered by several subsets of immune cells comprising CD1a^{neg} DCs, CD25⁺ T cells, and CD56⁺ regulatory cells.

CD4⁺CD25⁺FoxP3⁺ Tregs prevented the in vitro T cell priming to moderate/weak allergens, and depletion of human PBLs in CD25⁺ cells significantly increased specific T cell proliferation and IFN- γ secretion. CD56⁺ cells exerted an additional control of T cell priming since co-depletion of both CD56⁺ and CD25⁺ cells improved the magnitude of chemical-specific T cell activation. Finally, CD1a^{low} MDDCs were

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able to inhibit T cell activation obtained by allergen-pulsed CD1a^{high} MDDC. Moreover, we showed that uptake by DC of allergen-encapsulated nanoparticles significantly increased their activation status and their ability to prompt specific T cell activation. Hence, by combining the different strategies, i.e., depletion of CD25⁺ and CD56⁺ cells, use of CD1a^{high} MDDC, and nanoparticle encapsulation of allergens, it was possible to induce T cell priming to most of the moderate/weak allergens, including lipophilic molecules highly insoluble in culture media. Therefore, the present optimized in vitro human T cell priming assay is a valuable method to detect the sensitizing properties of chemical allergens.

Abbreviations

ACD	Allergic contact dermatitis
DCs	Dendritic cells
DMSO	Dimethyl sulfoxide
DNCB	2,4-Dinitrochlorobenzene
FITC	Fluorescein isothiocyanate
HCA	α -Hexylcinnamaldehyde
hTCPA	human T cell priming assay
ISs	Immunological synapses
mAb	monoclonal antibody
MDDCs	Monocyte-derived dendritic cells
MDLR	Mixed dendritic cell lymphocyte reaction
PBLs	Peripheral blood lymphocytes
PBMCs	Peripheral blood mononuclear cells
SLS	Sodium lauryl sulfate
TNBS	2,4,6-Trinitrobenzene sulfonic acid

7.1 Introduction

Allergic contact dermatitis (ACD) is a frequent skin inflammatory disease that develops upon repeated skin exposure of individuals to nonprotein chemicals, called haptens (Honda et al. 2013; Peiser et al. 2012; Vocanson et al. 2009). The priming of specific cytotoxic CD8⁺ T cells by hapten-presenting dendritic cells is regarded as the key event of the allergic process and the onset of the pathology (Vocanson et al. 2009; Akiba et al. 2002; Martin et al. 2000; Kehren et al. 1999).

T cell priming ensues from highly complex molecular and cellular processes that originate in the skin with chemical penetration and its fixation on extra- or intracellular proteins (Peiser et al. 2012) and lead to the emigration of skin resident (Kaplan et al. 2012; Bursch et al. 2007) or newly recruited DCs (Le Borgne et al. 2006), processing haptenated proteins, toward the draining lymph nodes (LNs). T cell priming is then initiated with the formation of transient but stable junctions (also referred to as immunological synapses (ISs)) (Dustin 2010;

Henrickson et al. 2008) at the interface between dendritic cells presenting haptenated-peptide MHC (hpMHC) complexes and naive T cell precursors, bearing T cell receptors (TCRs) with sufficient affinity/avidity for cognate hpMHCs.

Hence, throughout the travel of a hapten in the organism, a plethora of physico-chemical, biochemical, or immunological factors is susceptible to impact and finely tune the activation and differentiation fate of hapten-specific T precursors. A non-exhaustive list of the main immunological factors that can shape T cell priming and differentiation is as follows: (1) the duration and the strength of antigenic stimulus, which are directly correlated with the number of skin antigen-presenting cells (APCs) emigrating to LNs (Gorbachev and Fairchild 2010; Martin et al. 2003); (2) the density of pMHC per APCs (Henrickson et al. 2008), the nature of APCs (Gomez et al. 2012; Kaplan et al. 2012), and their functional state (Martin et al. 2008; Ring et al. 2010); (3) the antigen sensitivity of T cell precursors that pertain to TCR affinity/avidity for pMHC molecules (Speiser et al. 2008); and (4) finally, as T cell priming must not be envisioned as a bilateral dialogue between APCs and effector T cells, the collateral activation of T helper (Feau et al. 2012; Semmling et al. 2010) and/or regulatory T cells (Tregs) (Vocanson et al. 2010; Ring et al. 2010), as well as non- $\alpha\beta$ TCR⁺ lymphocytes such as $\gamma\delta$ T cells (Macleod and Havran 2011), NKT cells (Goubier et al. 2013; Semmling et al. 2010; Mattarollo et al. 2011), NK cells (Soderquest et al. 2011; Iyori et al. 2010), and even B cells (Nakashima et al. 2010), that trigger crucial loops of amplification or regulation on the functions of APCs and/or T cells.

Understanding the interplay between all these diverse parameters then represents a major challenge to develop a minimal *in vitro* assay that recapitulates the key event of the allergic process and allows for the characterization of chemicals endowed with sensitizing properties.

7.2 Difficulty in Obtaining Chemical-Specific T Cell Priming in Classical Autologous Mixed Dendritic Cells/Lymphocyte Reaction

In the past 2 decades, several groups have tested for the ability of haptens to prime naive human peripheral blood T cells in protocols, referred to as human T cell priming assays (hTCPA) (Martin et al. 2010). Approaches in these assays mainly consist of an autologous mixed DC-lymphocyte reaction (MDLR) using hapten-loaded DC as antigen-presenting cells and normal human peripheral blood leucocytes (PBL) as responding cells. The *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 5–7 day culture. 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, especially when clinically relevant molecules endowed with moderate/weak sensitizing

properties were evaluated (Moed et al. 2005; Rougier et al. 2000; Rustemeyer et al. 1999; Krasteva et al. 1996).

Possible reasons for the poor sensitivity of these assays are multiple: (1) the low uptake by DCs of molecules that are for the most part highly hydrophobic and therefore very difficult to dissolve in conventional culture media, (2) the inability of weak haptens to generate sufficient “danger signals” when delivered to DCs and to prompt their activation/maturation (Martin et al. 2011), and (3) the low frequency or affinity/avidity of hapten-specific T cell precursors in normal individuals (see the Chap. 8).

7.3 Depletion of Human Peripheral Blood Lymphocytes in CD25⁺ T Regulatory Cells Improves the In Vitro Priming of Chemical-Specific T Cells and Allows for the Development of a First-Generation hTCPA Able to Detect the Sensitizing Properties of Chemicals

Another explanation to these results is that in vitro priming of T cells may be repressed by CD4⁺CD25⁺FoxP3⁺ Tregs (that comprise up to 5 % of the peripheral CD4⁺ T cell pool), which are known to exert profound effects on inhibiting proliferation of naive T cells (Ohkura and Sakaguchi 2010). In this respect, studies in contact hypersensitivity reaction (CHS, the murine equivalent of ACD) to both strong and weak haptens have shown that hapten painting leads to a rapid and robust expansion of a highly suppressive Treg subset (that coexpressed FoxP3⁺ and Inducible Costimulator (ICOS)) that specifically suppresses hapten-reactive CD8⁺ T cells both in vivo and ex vivo (Vocanson et al. (2010) and data not shown). Alternatively, acute depletion of CD4⁺ T cells, CD25⁺ T cells, or of FoxP3⁺ Tregs in wild-type mice exposed to large variety of strong or weak sensitizers (such as perfumes, dyes, drugs, arnica tinctures) results in a dramatic increase in the number of hapten-specific effector CD8⁺ T cells (producing IFN- γ) in the LNs draining the sensitization site and exacerbation or development of skin inflammation (Rozières et al. 2010; Lass et al. 2008; Vocanson et al. 2006a) and data not shown).

Therefore, we postulated that hTCPA to haptens might be more efficient if Tregs were absent from the responding T cell population and tested in a standard autologous MDLR, whether depletion of the responding PBLs in CD25⁺ cells could increase the magnitude of hapten-specific T cell sensitization in vitro.

Monocyte-derived DC (MDDC) were incubated for 10 min with the strong/moderate haptens TNBS (2,4,6-trinitrobenzene sulfonic acid) and FITC (fluorescein isothiocyanate), with the clinically relevant weak fragrance allergen HCA (a-hexylcinnamaldehyde), or with the non-sensitizers DMSO (dimethyl sulfoxide) and SLS (sodium lauryl sulfate). After washing, haptenated DC 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 95 % depletion of CD25⁺ T cells (and in consequence >95 % depletion of FoxP3⁺ Tregs). Priming of hapten-specific T cells during the MDLR was determined by (1) analysis of cell proliferation following addition of ³HT for the last 8 h of culture and (2) titration of IFN- γ production in culture supernatants after addition of a sub-mitogenic dose of PHA for the last 15 h of culture, as previously described (Vocanson et al. 2006b; Cohen et al. 1974). Results were expressed as a stimulation index (SI) corresponding to the ratio of values recorded with hapten-pulsed versus unpulsed MDDC. Results (Fig. 7.1, Vocanson et al. 2008) showed that depletion of naive PBLs in CD25⁺ T cells improves the sensitivity of the primary sensitization assay and allows for detecting of the sensitizing properties of chemicals with moderate (FITC)/weak (HCA) allergenic properties. Indeed, classical primary autologous MDLR, i.e., using undepleted PBLs as responding cells, resulted in (1) a moderate proliferative response to TNBS (in 6/11 experiments) with IFN- γ production in 2/11 exp. only, (2) no proliferative response and IFN- γ production to FITC, and (3) proliferative response to HCA in 1/6 exp. only without IFN- γ production. In contrast, depletion of naive PBLs in CD25⁺ T cells increased the sensitivity of the MDLR inasmuch as (1) a strong T cell proliferation and IFN- γ production in response to TNBS was observed in almost all (12/13) experiments; (2) mild but significant proliferative responses to FITC and HCA were obtained in, respectively, 2/3 exp. (with IFN- γ production in 1/3 exp.) for FITC and 4/8 exp. (with IFN- γ production in 1/7 exp.) for HCA; and (3) the non-sensitizers DMSO and SLS did not induce any PBL proliferation or IFN- γ production.

Thus, depletion of PBLs in CD25⁺ T cells allows for improved in vitro priming of T cells against contact sensitizers but not against xenobiotics devoid of sensitizing properties (DMSO, SLS), confirming our primary hypothesis that regulatory cells prevent the priming/expansion of specific T cell precursors.

To date, the mechanisms by which Treg depletion boosts in vitro priming of hapten-specific T cells in primary sensitization assays remain to clarify. CD4⁺CD25⁺FoxP3⁺ Tregs harbor a wide arsenal of immunomodulatory mediators (Yamaguchi et al. 2011). Depending on the experimental model studied and their environment (lymphoid organs or peripheral tissues, inflammatory or steady-state conditions), Tregs could impair T cell responses through a large set of mode of action (Wing and Sakaguchi 2012) and modulate both the functions of antigen-presenting cells or the expansion program of T cells (Tang and Bluestone 2008). Hence, Tregs deliver inhibitory signals to DCs or T cells via their high expression of negative costimulatory molecules such as CTLA-4, the secretion of an inflammatory cytokines (IL-10, TGF- β , IL-35), the generation of immunosuppressive adenosine, or through cell-directed cytotoxicity and granzyme/perforin mechanisms (Yamaguchi et al. 2011). Of note, another prominent mechanism for Treg suppression, inherent to their high expression of the alpha chain of the IL-2 receptor (CD25), also consists in IL-2 deprivation in the pericellular environment of proliferating T effector cells (Pandiyani et al. 2007). Interestingly, in vivo imaging revealed that Treg depletion considerably affects DC behavior and profoundly

Chemical	Experiment number	Cell Proliferation						Cytokine secretion						
		Undepleted MDDC			CD25 ⁺ T cell-depleted MDDC			Undepleted MDDC			CD25 ⁺ T cell-depleted MDDC			
		3H-thymidine incorporation (cpm × 10 ³)		Stimulation Index	3H-thymidine incorporation (cpm × 10 ³)		Stimulation Index	IFN-γ secretion		Stimulation Index	IFN-γ secretion		Stimulation Index	
		Hapten-pulsed MDDC	Unpulsed MDDC		Hapten-pulsed MDDC	Unpulsed MDDC		Hapten-pulsed MDDC	Unpulsed MDDC		Hapten-pulsed MDDC	Unpulsed MDDC		
TNBS (1000 μg/ml)	1	1337 ± 1707	2551 ± 860	0.5	756 ± 359	40 ± 35	8.4	24	29	0.8	41	4	10.1	
	2	119 ± 420	368 ± 11	3.2	2192 ± 894	502 ± 216	4.4	6	6	1.0	37	7	5.3	
	3	1247 ± 2204	4700 ± 586	2.6	1770 ± 306	1365 ± 1410	12.8	76 ± 2	50 ± 22	1.5	307 ± 62	80 ± 40	4.5	
	4	2127 ± 233	1003 ± 337	1.6	824 ± 264	740 ± 229	8.7	43 ± 6	10 ± 4	3.2	12 ± 22	18 ± 6	4.5	
	5	160 ± 755	706 ± 134	2.6	812 ± 390	318 ± 157	2.4	102 ± 4	89 ± 17	1.1	260 ± 51	73 ± 22	3.6	
	6	864 ± 708	313 ± 406	2.8	618 ± 130	1630 ± 307	2.8	41	31	1.3	197	33	6.2	
	7	1405 ± 611	1245 ± 355	1.2	7660 ± 2130	215 ± 102	2.4	10 ± 3	9 ± 11	1	22 ± 11	7 ± 3	3	
	8	6760 ± 1208	4193 ± 540	1.6	6070 ± 1043	2722 ± 723	2.2	13	16	0.9	225	11	20.5	
	9	405 ± 1242	2213 ± 787	2.1	7440 ± 1178	3065 ± 1272	3.6	117	68	2	519	70	1.7	
	10	1141 ± 464	363 ± 78	3.1	9796 ± 282	3297 ± 1266	3	29 ± 7	23 ± 17	1.3	130 ± 6	4	4	
	11	1125 ± 115	1006 ± 262	1	704 ± 452	432 ± 418	1.7	144	155	0.9	19	14	1.4	
	12	nd	nd	-	1009 ± 602	502 ± 43	16.3	-	-	-	237	44	3.1	
FITC (3000 μg/ml)	13	nd	nd	-	3633 ± 1281	1130 ± 547	4.7	nd	nd	-	222	20	11.1	
	6	2930 ± 885	1133 ± 406	0.9	1056 ± 488	318 ± 157	3.7	87 ± 23	117 ± 47	0.7	222 ± 29	202	1.1	
	8	4930 ± 2011	4193 ± 540	1.4	2838 ± 530	1630 ± 307	1.2	16	31	0.5	22	32	1.6	
	1	663 ± 228	2361 ± 600	0.25	498 ± 71	250 ± 45	5.5	5	nd	6	8	nd	7	0.4
	2	467 ± 152	368 ± 11	1.1	570 ± 227	502 ± 216	1.1	10 ± 1	9 ± 11	1	2	7 ± 3	1	2.2
	7	1871 ± 615	1245 ± 355	1.5	275 ± 71	315 ± 102	0.9	30 ± 1	9 ± 11	1	153	30	0.8	
HCA (130-400 μg/ml)	9	2148 ± 616	2233 ± 787	1	6262 ± 1181	2065 ± 1272	2	48	48	1.3	130 ± 6	1	1.7	
	10	76 ± 64	364 ± 78	2.1	1123 ± 101	320 ± 126	3.4	23 ± 20	23 ± 17	1.1	218 ± 91	14	0.7	
	11	1213 ± 149	1006 ± 262	1.1	81 ± 626	432 ± 418	2	136	155	0.9	31	44	0.7	
	12	nd	nd	-	156 ± 43	102 ± 43	1.5	nd	nd	-	18	20	0.9	
	13	nd	nd	-	1501 ± 964	113 ± 587	1.3	nd	nd	-	203 ± 44	202	1	
	5	690 ± 48	706 ± 136	0.9	248 ± 57	318 ± 157	0.7	100 ± 21	119 ± 47	0.8	22	32	0.7	
DMSO (1000 μg/ml)	6	3052 ± 383	1133 ± 406	1	1753 ± 485	1630 ± 307	1.1	10	31	0.3	22	32	0.7	
	8	5347 ± 1581	4193 ± 540	1.3	3608 ± 247	2722 ± 43	1	14	nd	16	0.9	5	11	0.5
	1	101 ± 125	2361 ± 600	0.4	172 ± 308	50 ± 45	1.9	nd	nd	-	nd	nd	-	
	3	366 ± 201	368 ± 11	1	979 ± 230	502 ± 216	1.9	nd	nd	-	nd	nd	-	
SLS (4-40 μg/ml)	7	nd	nd	-	125 ± 34	102 ± 43	1.2	nd	nd	-	17	48	0.6	
	12	nd	nd	-	125 ± 34	102 ± 43	1.2	nd	nd	-	17	48	0.6	

Fig. 7.1 In vitro T cell priming to contact sensitizers is improved in the absence of CD25⁺ cells. Dendritic cells were generated from 6-day cultures of monocytes (isolated from peripheral blood of healthy donors) in complete RPMI medium supplemented with 10 % FCS and recombinant human GM-CSF and IL-4. For each experiment, monocyte-derived dendritic cells (MDDC) were then haptenized with 1000 μ g/ml TNBS, 300 μ g/ml FITC, 130–400 μ g/ml HCA, 1,000 μ g/ml DMSO, or 4–40 μ g/ml SLS for 10 min at 37 °C. After extensive washing, 4–8 $\times 10^5$ hapten-pulsed and unpulsed MDDCs were cultured for 5–7 days with 2 $\times 10^5$ autologous lymphocytes (depleted or not in CD25⁺ cells using anti-CD25 mAb-coated microbeads and negative selection columns (Myltenyi Biotech, Bergish Gladbach, Germany)). The proliferative responses were assayed by ³HThymidine incorporation (1 μ Ci/well) for the last 8 h of culture. INF γ secretion was measured by ELISA assay in the supernatant of cultures restimulated for the last 15 h with 2 μ g/ml of PHA. Lymphoproliferation and INF γ secretion induced by hapten-pulsed MDDC were compared to that obtained with unpulsed MDDC, and results are expressed as stimulation index (SI): response recorded for hapten-pulsed MDDC/response recorded for unpulsed MDDC. Results are considered positive for SI >2 (underlined in the figure). nd not determined

increases their ability to initiate long-standing and stable ISs with naive T cells (Tang and Krummel 2006). Similarly, others studies reported that DCs, collected in the LNs of animals depleted by an anti-CD25⁺ mAb, display a much more mature phenotype (increased levels of the costimulatory CD86 marker and/or decreased level of the inhibitory B7-H3 molecule) (Schildknecht et al. 2010; Mahnke et al. 2007). Those two observations then suggest that DCs operating in Treg-depleted environment are intrinsically more prone to activate naive T cells and stimulate their proliferation/differentiation. Alternatively, we cannot exclude that additional inhibitory signals are brought into cultures of undepleted PBLs by a potent hapten-induced activation of Tregs. Indeed, we detected a significant enlargement in the percentage of CD25⁺FoxP3^{high} Tregs expressing the cell cycle protein Ki67 in cultures of undepleted PBLs stimulated with TNBS-pulsed MDDCs versus unpulsed cultures (data not shown). Are the proliferating CD25⁺FoxP3^{high} Tregs operate in an antigen-dependent manner, similarly to our previous results in mice (Vocanson et al. 2010), or are they polyclonal Tregs that are activated by the inflammatory signals delivered by haptens? Both antigen-specific and polyclonal Tregs (encompassing a very low number of specific cells and mainly cells dedicated

to respond to self-antigen presentation) were reported to modulate immune responses to exogenous antigens and CHS to hapten (Vocanson et al. 2010; Ring et al. 2006). Nevertheless, the two subpopulations would not mediate their biological functions using the same mechanisms. Antigen-specific Tregs operate early in the immune response and completely inhibit T effector cell expansion and differentiation via inhibition of DC functions, while polyclonal Tregs operate in later stage and appear to modulate differentiation and trafficking of effector T cells (Shevach 2011).

7.4 Obstacles to Overcome in Order to Increase the Efficiency of In Vitro T Cell Priming to Contact Sensitizers

While we succeeded in detecting significant proliferative responses to moderate contact sensitizers (FITC or HCA) in the first-generation hTCPA where CD25⁺ Tregs are absent from the responding PBL population, our assay failed to give positive results in about one out of two donors tested (similar data were recently recorded for a large panel of moderate/weak contact sensitizers) and was not sensitive enough to activate weak sensitizer-specific T cells. Several hypotheses could explain the difficulty in obtaining an optimal and consistent T cell activation in primary MDLR: (1) the frequency or affinity/avidity of hapten-specific T cell precursors may be too low in some individuals to be detected in the assay, (2) regulatory mechanisms other than CD25⁺ Tregs may control specific T cell priming, and (3) the delivery of chemicals to DCs may not be optimal, the more so since most of contact sensitizers are hydrophobic and cannot be easily dissolved in culture medium.

In order to improve the efficiency of in vitro T cell priming, we tested the two latter hypothesis and obtained significant improvement in the ability to detect the sensitizing properties of chemicals.

7.5 Second-Generation hTCPA: In Vitro Priming of Allergen-Specific T Cells Requires Allergen Uptake by CD1a⁺ Dendritic Cells and Is Hampered by Both CD25⁺ and CD56⁺ Regulatory Cells

7.5.1 CD56⁺ Cells Prevent T Cell Priming

We demonstrated that along with CD4⁺CD25⁺FoxP3⁺ Tregs, CD56⁺ cells (that comprise both NK and T cell subsets) present in responding PBLs exert a

supplementary control on T cell responses. Indeed, CD56⁺ cell depletion in responding PBLs modestly increased T cell priming to haptens, but when cells were included in CD25⁺ cell-depleted cultures, they actively restrained hapten-induced T cell proliferation and IFN- γ -secretion, notably because those cells (and respective NK and T cell subsets) efficiently kill chemical-pulsed MDDCs. Hence, double CD25⁺/CD56⁺ cell depletion in responding PBLs enhanced very significantly T cell responses and detection of a large panel of standard contact sensitizers (data not shown).

7.5.2 CD1a Positive DC Is the Most Efficient DC Subset for In Vitro T Cell Priming

Next, we also showed that T cell priming critically depends on the phenotype/functions of MDDCs used in the MDLR. Indeed, we observed that, depending on the donors, MDDCs generated from blood monocyte fractions varied substantially for their expression of CD1a marker (with distinction between CD1a^{neg} and CD1a^{pos} subsets). Respective CD1a^{neg} MDDC subset displayed less mature phenotype (with persistent expression of the CD14⁺ monocyte marker) and poor stimulatory capabilities compared to their CD1a^{pos} counterpart. Importantly, addition of CD1a^{neg} MDDC to CD1a^{pos}-stimulated cultures abrogated TNBS proliferation and type-1 differentiation (data not shown), demonstrating that precious attention must be brought to the type of MDDCs that is used in T cell priming assays.

7.5.3 Efficient Uptake of Hydrophobic Chemicals by DC Increases Their Ability to Prime T Cells

Finally, we also demonstrated that T cell priming to hydrophobic molecules such as HCA or DNCB is significantly hampered by their DC uptake. Indeed, the use of encapsulated chemicals in nanoparticles to pulse MDDCs significantly increased their activation state (as measured by their increased expression of the maturation markers CD86, CD83, HLA-DR), as well as their ability to prompt T cell responses to those two chemicals (but not to the non-sensitizer methyl salicylate).

7.5.4 Toward a Second-Generation hTCPA

Of great importance, combining depletion of cells endowed of regulatory functions (both CD25⁺ and CD56⁺) and nanoparticle strategies to increase the allergen uptake

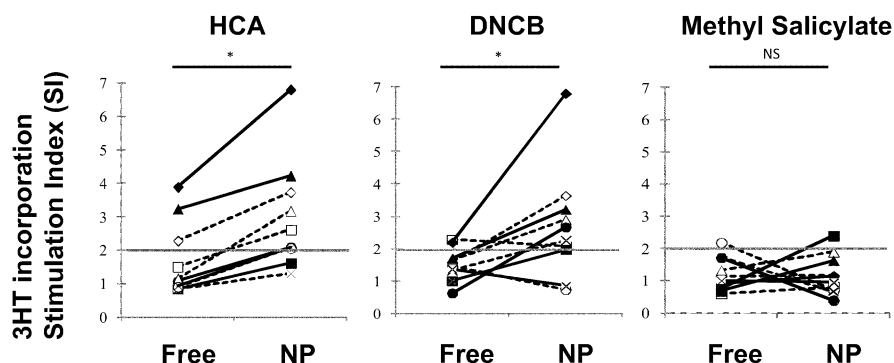


Fig. 7.2 Combining depletion of cells endowed with suppressive activity ($CD25^+/CD56^+$) and nanoparticle encapsulation of chemicals allows for sensitive screening of contact sensitizers in quasi-all the donors tested (A). MDDCs were pulsed for 15 min with various concentrations of free- or nanoparticle-encapsulated chemicals (20 (—), 40 (...) $\mu\text{g/ml}$ HCA; 10–20 (—), 40–50 (...) $\mu\text{g/ml}$ HCA; 10 (—), 50 (...) $\mu\text{g/ml}$ methyl salicylate). MDDCs pulsed with RPMI medium or empty nanoparticles were used as controls. 16×10^4 DC were then irradiated and cultured for 5 days with 2×10^5 autologous PBLs depleted in $CD25^+/CD56^+$ cells. Proliferative responses were assayed by ^3H thymidine incorporation for the last 8 h of culture. Responses induced by chemical-pulsed MDDCs were compared to that obtained with control MDDCs and results were expressed as stimulation index (SI): response recorded for chemical-pulsed MDDCs/response recorded for control MDDCs. Results were considered positive for $SI > 2$. Responses recorded for five different donors (round, square, diamond, triangle, cross) per chemicals are shown

by DC allowed the detection of specific T cell priming to HCA and DNCB (but not to the non-sensitizers methyl salicylate) in about all the donors tested (4 out of 5) (Fig. 7.2). Therefore, the second-generation hTCPA can be a valuable method to detect the sensitizing properties of chemicals.

7.6 Conclusions

So far, primary T cell sensitization assays had proved poorly robust for the screening of weak contact sensitizers, suggesting that some important signals relative to ACD response are missing in these assays. We report that T cell priming to haptens, notably to some weak sensitizers, can be significantly improved by eliminating from PBLs several cell subsets that suppress T cell proliferation and differentiation. Hence, we demonstrated that $CD4^+CD25^+FoxP3^+$ Tregs (as expected) but also $CD56^+$ cells (comprising both NK and T cell subpopulations) and $CD1a^{neg}$ MDDCs deliver potent inhibitory signals that prevent or limit T cell expansion. Thus, hTCPA protocols using naive T cells that are depleted of both $CD4^+CD25^+FoxP3^+$ and $CD56^+$ cells will undoubtedly provide progress for the development of these assays (Martin et al. 2010; Richter et al. 2013; Vocanson, manuscript in preparation). Nevertheless, it appears crucial to combine such

strategy to the use of MDDCs endowed with potent stimulatory capacities and, more importantly, to the use of synthetic vectors that can boost chemical uptake (notably the less soluble in conventional culture media) and MMDC activation. That positive hTCPA responses to haptens, such as HCA or DNCB, were obtained for quasi-all the donors tested indicates that such simple immunological assay represents undeniably a good alternative to test for the potential immunogenic properties of haptens, especially those endowed with weak sensitizing properties.

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