INMUNE MECHANISMS INVOLVES IN HYPERSENSITIVITY REACTIONS TO HELIXONE HEMODIALYSIS MEMBRANES

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INTRODUCTION AND AIMS

•Hemodialysis (HD) sessions involve some risk of adverse hypersensitivity reactions as large amounts of blood are in contact with different synthetic materials. Our aim was to study the mechanisms of the allergic or "pseudoallergic" reactions to synthetic helixone (HX) dialysis membranes in "HX-allergic" patients who tolerated cellulose triacetate (CTA) membranes. As only exceptionally we had the opportunity of studying the acute phase of the allergic reaction we designed an "ex-vivo" approach to compare immune responses to both HX and CTA.

METHODS

•Ten patients (table 1) with adverse reactions to HX and 8 control non-allergic patients in hemodialysis were studied. 50 ml of blood was collected into heparin tubes. Ex-vivo HD were performed on experimental external circuits with low or high priming volumes and pediatric membranes (Fx-Paed helixone 0.2 m2 and Sureflux cellulose triacetate 30L 0.3 m2). Pre-dialysis and post-dialysis samples were collected. Serum tryptase levels, basophil degranulation (%HLA-DR-CD123+CD63+ leukocytes), and T cell activation (CD69 expression on CD4+ and CD8+ subpopulations) were analyzed by flow cytometry.

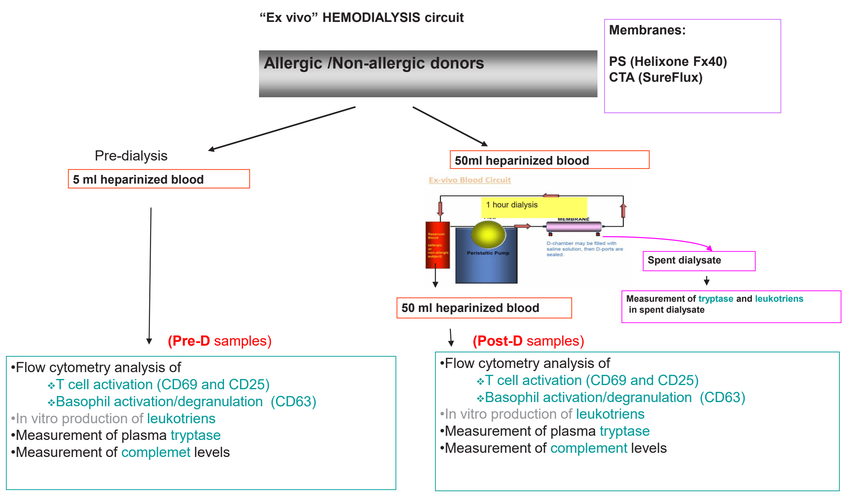


Table 1. Demographic data of patients with adverse reactions during HD

Patient	Age (Years)	Sex	Months*	Problem membrane	Symptoms	Acute samples
P1	67	M	38	Helixone	Hypotension, Dyspnea, Desaturation	No
P2	65	M	0	Helixone	Hypotension, Dyspnea, Desaturation	No
P3	86	F	0	Helixone	Hypotension, Dyspnea, Desaturation	No
P4	64	M	4	Polinephrone	Dyspnea, Desaturation	No
P5	72	M	6	Helixone	Hypotension, Dyspnea, Desaturation	No
P6	84	F	5	Helixone	Hypotension, Pruritus,Abdominal pain	No
P7	53	F	8	Helixone	Hypotension, Pruritus	No
P8	73	M	0	Helixone	Hypotension, Dyspnea, Desaturation	No
P9	74	F	0.5	Helixone	Abdominal pain, Rash ***	No
P10	84	M	0	Helixone	Dyspnea, Desaturation	**YES**
P11	53	F	1	Helixone	Dyspnea, Pruritus	**YES**
P12**	60	F	0	**HEPARINE Rx****	Dyspnea, Desaturation	**YES**

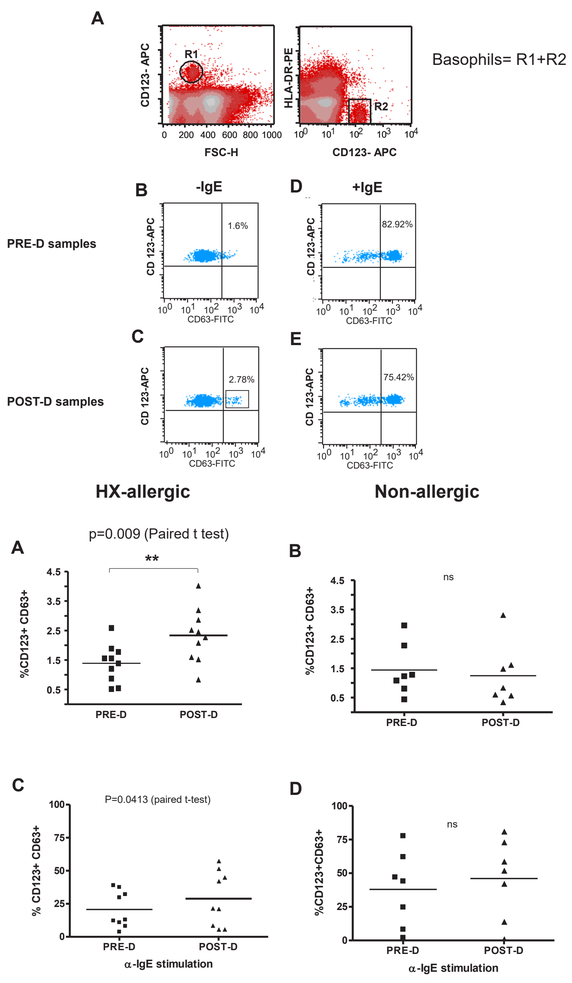
*Time period using a polysulfone hemodialyzer previous to the HS reaction ;**P12 suffered a reaction to heparine while using a Helixone dialyzer; ***2nd rx

RESULTS

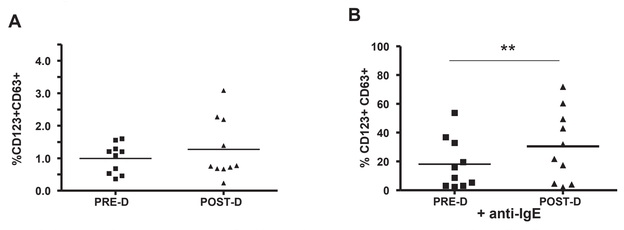
•Basal serum tryptase levels were higher in HX-allergic patients as compared to control donors (12.47±5.67 *vs* 10.4±3.13 ng/ml). Basophils (Fig. 1) showed increased degranulation (mean % CD63+:2.33±0.9 vs 1.31±0.63; p= 0.009), and T cells (CD4+ and CD8+) from HX-allergic patients (Fig. 2) showed significantly increased activation after contact with Hx membranes primed with low volumes of saline (% CD4+ CD69+ T cells: 8,3±4.6 vs 3.7±1.8 %; p= 0.048). No activation was detected in leukocytes from non-allergic patients. Membrane priming with high volumes of saline abrogated activation of basophils and T cells. However, basophils from allergic donors showed significantly higher responses to FceR stimulation after contact with HX membranes.

•Acute samples from 2 HX-allergic patients were analyzed. Samples from a 3rd reaction to heparin were also studied for comparative purposes. Basophil and T cell activation was detected in acute samples (Table 2). Serum tryptase levels were higher in acute samples compared to basal levels, suggesting activation of mast cells and basophils. Complement levels (C3 and C4) were specifically decreased in acute samples from HX-allergic patients (Table 3). As the reactions typically occurred after 15'-20' after initiation of HD, complement levels were measured in 3 control patients after 20' of an uneventful HD session (Table 4). C3 and C4 degradation was higher in acute samples that in control donors (38.15% vs 7.68% of C3 and 32.83% vs 4.31% of C4).

Fig 1. Basophils

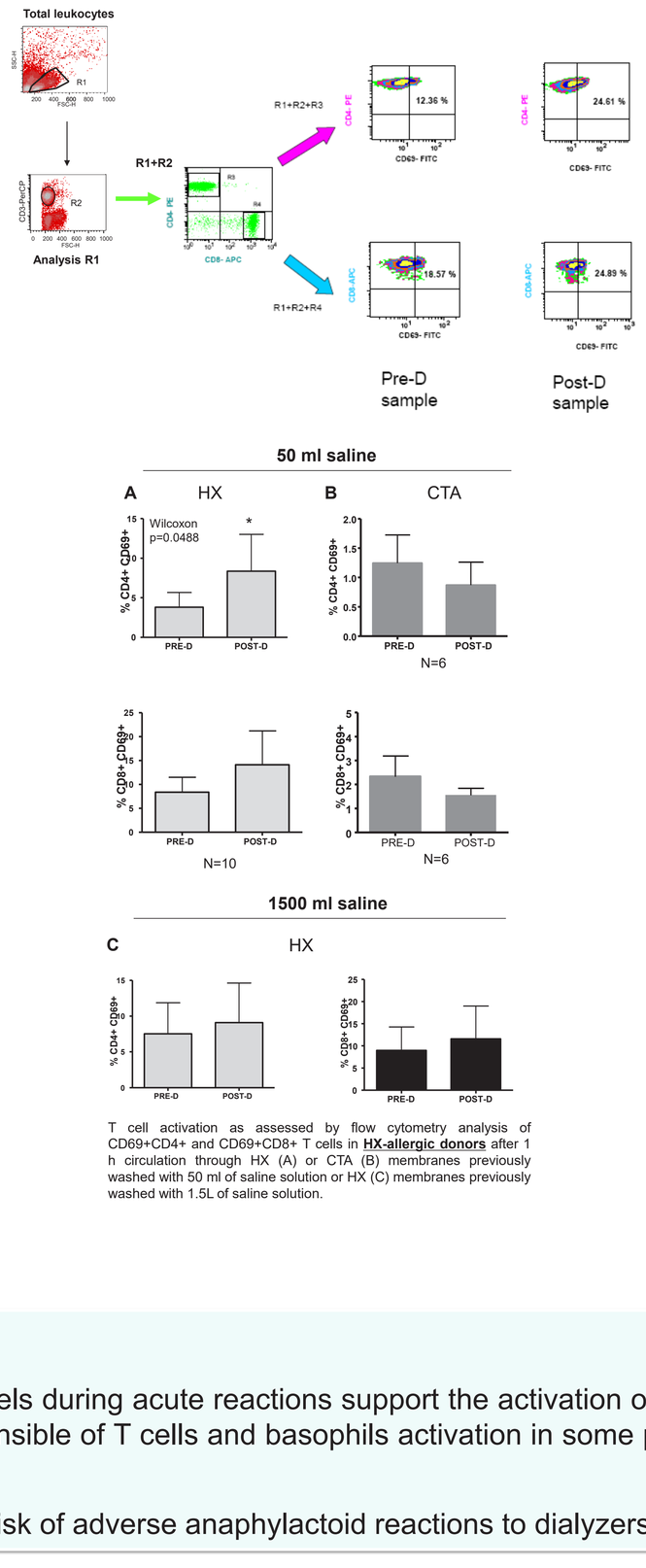


Basophil degranulation in allergic or B) non-allergic donors upon 1 hour circulation through **HX membranes** previously washed with 50 ml saline was analyzed by flow cytometry as the % of CD63+ CD123+HLA-DR- leukocytes. C and D) After undergoing the HX ex-vivo circuit, blood cells from allergic or non-allergic donors were stimulated with anti-IgE antibodies and basophil degranulation was analyzed. Data from 10 allergic donors and 7 non-allergic donors are shown. ** p=0.009 (paired t-test); n.s: not significant.



Basophil degranulation in blood from 10 HX-allergic donors was analyzed before (Pre-D samples) and after (Post-D samples) 1 hour circulation through HX membranes previously washed 1,5 L of saline solution. B) Basophil degranulation was analyzed in the same blood samples after further IgE receptor stimulation. ** p=0.0024 (paired t-test)

Fig 2. Leukocytes



T cell activation as assessed by flow cytometry analysis of CD69+CD4+ and CD69+CD8+ T cells in **HX-allergic donors** after 1 h circulation through HX (A) or CTA (B) membranes previously washed with 50 ml of saline solution or HX (C) membranes previously washed with 1.5L of saline solution.

Acute samples

Table 2. Basophil and T cell activation in acute samples of Hx-allergic patients

Patients	T cell activation	Acute sample	2h post-rx	24h post-rx
P9	% CD123+CD63+	61.03	1.27	3.29
	% CD4+CD69+	76.63	82.88	1.37
	% CD8+CD69+	31.31	34.74	0.88
P11	% CD123+CD63+	2.83	N.D.	1.90
	% CD4+CD69+	0.66	N.D.	1.11
	% CD8+CD69+	3.81	N.D.	4.81
P12 (HEPA)	% CD123+CD63+	1.98	0.89	0.37
	% CD4+CD69+	46.70	62.9	0.33
	% CD8+CD69+	83.79	88.4	0.69

N.D.: No data

Table 3. Levels of tryptase and complement in acute samples

	P9		P11		P 12 ((Heparin)	
	Acute sample	24h post-reaction	Acute sample	24h post-reaction	Acute sample	24h post-reaction
Tryptase (ng/ml)	32.2	25.0	7.73	5.97	4.85	
C3 (ng/ml)	73.6	119.0	77.4	109	129	126
C4 (ng/ml)	26.8	33.1	17	24.9	29.9	31

Table 4. Serum complement levels in control and HX-Allergic donors during HD

Patients	Complement C3 (ng/ml)		%C3 degraded at 20'	Complement C4 (ng/ml)		%C4 degraded at 20'
Non-allergic	Basal levels	post-D 20'		Basal levels	post-D 20'	
C1	119.0	103.0	13.45		26.6	0.75
C2	110.0	109.0	0.91	27.9	26.3	5.73
C3	115.0	105.0	8.70	35.6	33.3	6.46
Mean			7.68			4.31
Allergic	Basal levels	Acute sample		Basal levels	Acute sample	
P9	119.0	73.6	38.15	39.9	26.8	32.83
P11	109.0	77.4	28.99	24.9	17.0	31.70

CONCLUSIONS

•Basophil degranulation in HX-allergic patients and serum tryptase levels during acute reactions support the activation of mast cells and basophils during hypersensitivity reactions to HX membranes. A leachable component of the membranes may be responsible of T cells and basophils activation in some patients. Complement activation may also participate in activating mast cells and basophils.

•Adequate priming of membranes seems to be important to lower the risk of adverse anaphylactoid reactions to dialyzers

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Rafael Sanchez-Villanueva

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