

The Effect of Thermal Treatment of Immunoglobulin E as Measured with Competitive and Noncompetitive Assays: Is Circulating Immunoglobulin E Serologically Heterogenous?¹

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Abstract. Using two-double antibody assays (DA) and a noncompetitive assay (PRIST), we measured in 20 atopic and 10 normal sera the total IgE before and after its heating at 56°C for 30-120 min. After heating the sera or radiolabeled IgE, DA detects a reproducible increase in total IgE associated with a large intersubject variability of the inhibition curves. On the contrary, heated total IgE measured with PRIST decreases and there is no intersubject difference in the observed change. In conclusion, DA assays of IgE reveal a heterogenous response to heating which might be related to interindividual differences in the tertiary/quaternary structure of the D Σ domain of Fc Σ

The heating of human [1, 2] or animal [3, 4] sera containing skin-sensitizing antibodies (i.e., specific IgE) decreases the concentration of these antibodies, as measured by passive cutaneous sensitization or noncompetitive serologic assays. The purpose of the present work was to measure the effect of heating human sera on IgE measurements obtained by means of competitive and noncompetitive radioimmunoassays. We wondered whether the thermal inactivation of IgE is homogenous across different individuals.

We studied 30 sera, 10 from normal and 20 from atopic subjects, with total IgE > 200 U/ml but less than 1,000 U/ml. Undiluted samples along with 1/10 and 1/100 dilutions of these sera were tested for total IgE before and after being heated at 56°C for 30, 60 and 120 min. IgE was measured by two methods.

The double antibody method [5] followed the steps proposed by Gleich et al. [6] and was capable of detecting small changes in the inhibition curves produced by various alterations in the reactants. Briefly, 0.1 ml of various dilutions of the sera to be tested and the international standard serum were added to 1.0 U radiolabeled myeloma IgE (IgE_{ND} and IgE_{PS} kindly provided by Drs. S.G.O. Johanssen and K. Ishizaka, respectively), 0.1 ml antihuman myeloma IgE, Fc Σ

specific, diluted 1:10,000 (anti-IgE_{PS}, Hyland Laboratories) or 1:2,000 (anti-IgE_{ND}, Cappel Laboratories), and phosphate-albumin buffer to bring the resulting volume to 0.4 ml. The tubes were incubated overnight at 4°C. For coprecipitation, we added 0.2 ml second antibody and 0.2 ml normal sheep or goat serum diluted 1:90. After another overnight incubation, the tubes were centrifuged, the supernatant decanted and the radio-activity in their precipitate measured in the gamma counter. All measurements were performed in triplicate. PRIST (Pharmacia Diagnostics) was used as the noncompetitive assay, following the indications of the manufacturer. The mean values of IgE were compared by using Student's t test for paired or unpaired observations as required.

In the first experiment, total IgE was measured with two competitive assays, ¹²⁵I-IgE_{ND} - anti-IgE_{PS} + cold IgE and ¹²⁵I-IgE_{PS} + cold IgE [5], before and after heating either the tracer or the subjects' sera at 56°C for 30 min. The reproducibility was checked by running the same experiment 48 h later. The results were compared to those obtained with PRIST at the same temperature but after 60 min of heating. Preliminary experiments with sera heated at 56°C have shown that for all dilutions, the first significant changes in IgE were detected by the DA method 30 min and by PRIST 60 min after heating. Regardless of the assay used, increasing the duration of heat-

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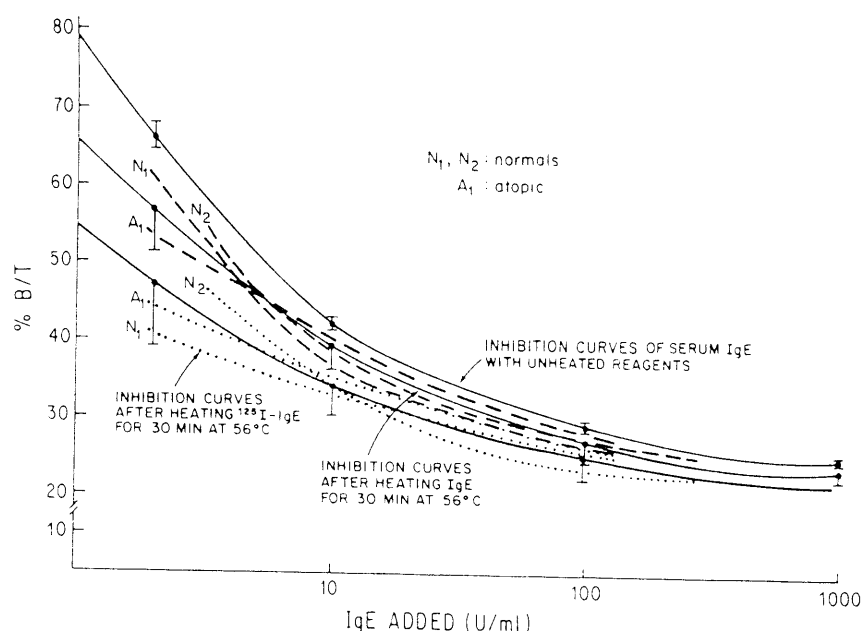


Fig. 1. Effect of heating the tracer (^{125}I -IgE) or the serum IgE itself on the inhibition curves obtained with the latter protein. The bars indicate the standard deviation of all inhibition curves of IgE before and after thermal treatment of the cold (...) or hot (----) immunoglobulin. As exemplified in this figure, the individual inhibition curves are no longer parallel and the atopic (A) and normal (N) sera react in the same way.

ing up to 120 min did not lead to further alterations in the concentration of this immunoglobulin.

Both DA assays revealed that the concentration of heated IgE, read off the calibration curve with unheated standard, increased in all serum concentrations tested. This increase was reproducible for each competitive assay ($p > 0.05$ for repeat tests), of similar magnitude across assays ($p > 0.05$) and significantly larger at $1/10$ or $1/100$ dilutions than the change in undiluted samples ($p < 0.05$; table I). When the monoclonal tracer was heated before its mixture with the first antibody and the various dilutions of unheated serum IgE, the concentration of the later protein was reproducibly higher than its concentration measured previously with unheated tracer ($p < 0.05$; fig. 1). The increase in IgE concentration involved much more the $1/100$ dilution than the $1/10$ dilution or the undiluted serum sample: ≈ 10 -fold vs. 4-fold or ≈ 3.5 -fold, respectively (table I). Unlike the inhibition curves obtained with unheated samples, the individual inhibition curves with heated IgE or heated tracer were not only shifted to the left but also became nonparallel, fanning out at lower concentrations (fig. 1).

A shift to the left of the inhibition curve obtained with thermally treated cold or hot IgE leads to an apparent increase in the concentration of this immunoglobulin and consequently reflects a relative decrease in the concentration of the first antibody. It is well established that, as the concentration of the first anti-

body decreases the inhibition curve of the ligand is shifted to the left, more so at low than at high concentrations (i.e. a nonparallel left shift as compared to the initial curve) [7]. That the shift in the inhibition curves with heated IgE is heterogenous across subjects, the curves assuming different slopes (fig. 1), implies that the amount of 'new' IgE sites appearing after thermal treatment varies across individuals. These new sites are actually public since two different DA assays produce similar results and the shift of inhibition curves is different than that observed with myelotropic determinants [5]. Furthermore, these public sites seem to be equally well represented in atotics and normals because there is no consistent difference between these two groups of subjects in terms of effect of heating (fig. 1). After 60 min of heating at 56°C , the total IgE measured by PRIST decreased from $460.1 \text{ U/ml} \pm 225.7$ (undiluted), $48.9 \text{ U/ml} \pm 30.1$ (diluted $1/10$) and $5.7 \text{ U/ml} \pm 2.7$ (diluted $1/100$) to $245.7 \text{ U/ml} \pm 111.9$, $25.1 \text{ U/ml} \pm 16.2$ and less than 5.0 U/ml , respectively. The intersubject variation of the thermally treated IgE (10%) was close to the within day variability of the IgE measurement with PRIST (8%).

Heating can produce various conformational modifications in the IgE molecule: Changes in the tertiary/quarternary structure of D Σ 2 domain due to the unfolding of this portion of IgE molecule [8], polymerization [9] or exposure of new sites previously occupied by anti-D Σ 2 autoantibody [10]. Polymeriza-

Table I. Effect of heating the cold and radiolabelled IgE on serum IgE measurements

Thermal treatment		IgE measurement, U/ml			
Treatment	Dilution	¹²⁵ I-IgE _{PS} + anti-IgE _{ND}		¹²⁵ I-IgE _{ND} + anti-IgE _{PS}	
		1st run	2nd run	1st run	2nd run
Unheated	UD	420.7 ± 217.7	455.1 ± 230.7	450.2 ± 220.7	465.4 ± 231.4
	1/10	45.8 ± 29.4	50.0 ± 29.7	52.4 ± 30.1	54.1 ± 31.2
	1/100	4.9 ± 3.0	5.2 ± 3.1	5.5 ± 3.4	6.1 ± 3.1
Heated IgE (56°C for 30 min)	UD	620.4 ± 310.7*	635.0 ± 321.3*	671.0 ± 339.9*	689.7 ± 347.7*
	1/10	70.1 ± 41.4*	74.7 ± 44.1*	77.0 ± 46.4*	81.3 ± 51.3*
	1/100	9.8 ± 8.3*	12.1 ± 11.8*	13.1 ± 12.4*	14.0 ± 12.9*
Heated ¹²⁵ I-IgE (56°C for 30 min)	UD	1621.7 ± 625.4**	1708.0 ± 827.4**	1780.5 ± 840.1**	1845.0 ± 861.4**
	1/10	200.4 ± 110.4**	224.1 ± 121.9**	241.3 ± 131.0**	258.4 ± 134.3**
	1/100	41.4 ± 21.4**	45.4 ± 24.3**	49.5 ± 27.2**	52.4 ± 29.3**

UD = Undiluted.

* Statistically significant difference ($p < 0.05$) when compared to the corresponding unheated sample.** Statistically significant difference ($p < 0.05$) when compared to measurements of both unheated and heated serum IgE. For each dilution, there is no significant difference within runs with the same assay or across runs with a different competitive test.**Table II.** Effect of switching the order of incubation of first antibody with unheated ¹²⁵I-IgE or heated IgE

IgE dilutions	IgE (U/ml) after switching the order of incubation with anti-IgE					
	2-hour incubation		4-hour incubation		4-hour incubation	
	label first (1)	IgE first (2)	label first (3)	IgE first (4)	label first (5)	IgE first (6)
Undiluted	790.4 ± 389.4	870.0 ± 401.0	810.4 ± 370.5	889.4 ± 390.4	900.4 ± 450.4	1010.0 ± 484.4
Dilution 1/10	84.3 ± 61.3	93.2 ± 63.5	87.3 ± 62.4	96.3 ± 66.4	99.4 ± 69.4	120.0 ± 75.3
Dilution 1/100	16.5 ± 15.4	18.1 ± 15.9	17.1 ± 15.5	19.1 ± 16.4	19.8 ± 16.9	21.8 ± 18.9

In all experiments serum was heated at 56°C, for 1 h in the first 4 experiments (1-4) and for 2 h in the last 2 experiments (5, 6). All IgE measurements were performed with ¹²⁵I-IgE_{ND} + anti-IgE_{PS} + cold IgE.

ion is expected to decrease rather than increase the available binding sites of IgE [9]. A dissociation of auto-IgE antibodies from IgE produced by thermal treatment [10] could account for the increase in IgE after heating the sera but not the radioligand. Indeed, unlike the former, the latter IgE is a purified protein, without any contaminating IgA, IgG or IgM [5]. In the experiments with heated ligand and unheated sera, a transfer of autoanti-IgE antibodies from native to labeled IgE, although possible, would in the end decrease rather than increase the amount of tracer. Another experiment indicates that although the affinity of the first antibody may be minimally higher for heated than for unheated IgE, it fails to achieve statistical significance.

In this second experiment we determined the differences in the binding affinity for anti-IgE antiserum between heated and unheated serum IgE by incubating sequentially the first antibody with either the native or the thermally inactivated immunoglobulin. The results (table II) indicate that the anti-IgE antibody does not have a higher affinity for heated IgE than for unheated radiolabelled IgE, and thereby it cannot explain the left shift of the inhibition curves reported in this study.

It thus appears that thermal treatment of human IgE unfolds the D Σ 2 domain [8] and via changes in the tertiary/quaternary structure [8] exposes new, public IgE-binding sites in a manner that varies from subject to subject. From this point of view, the D Σ 2

domain appears to be not only functionally [11-13] but also serologically heterogeneous. These new public sites seem to interfere with (a) the binding of IgE to mast cells, since thermally treated IgE has less skin-sensitizing activity than native IgE, and (b) the determination of IgE by noncompetitive assays, since these methods indicate that the concentration of this protein decreases after heating [10, 14].

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