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## Idiotypic and anti-idiotypic elastin autoantibodies: Implications for IVIg and pregnancy loss

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### Abstract

**Problem:** The aim of this study was to investigate anti-elastin and anti-anti-elastin autoantibodies in intravenous immunoglobulin (IVIg) lots as an attempt to further explain the effect of IVIg in recurrent pregnancy loss (RPL).

**Method of Study:** Serum samples of 10 female patients with RPL and 10 healthy subjects were tested for anti-elastin autoantibodies and used in competitive inhibition studies. A total of 44 IVIg lots (ZLB Behring, Switzerland) were tested for anti-elastin and anti-anti-elastin idiotypes. One way analysis of variance (ANOVA) and Least Significant Difference (LSD method) were used for statistical analysis of differences between the lots.

**Results:** Serum anti-elastin IgG autoantibodies were significantly higher in the study group, compared to the controls. In all lots anti-elastin IgG antibodies were identified. All lots (except two of them) showed similar dose-dependent inhibition of serum anti-elastin activity by anti-elastin anti-idiotypes in IVIg.

**Conclusions:** Anti-elastin IgG autoantibodies were increased in patients with RPL — a finding which needs further explanation. Anti-elastin and anti-anti-elastin idiotypes were identified in different IVIg lots. The presence in IVIg of anti-idiotypes against anti-elastin autoantibodies from patients' sera could be an additional mechanism of the beneficial effect of IVIg in reproductive failure.

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### 1. Introduction

The therapeutic use of intravenous immunoglobulin (IVIg) is based on two fundamental strategies: replacement therapy (for primary or secondary humoral antibody deficiency) and immunomodulation (for autoimmune diseases). Multiple immunomodulating effects of IVIg in autoimmune disorders can be summarized in the following basic mechanisms: anti-

idiotype interactions, Fc receptors blockade, effects on the cytokine network, and increased catabolism of IgG [1–3].

Normal serum contains IgG, IgM, and IgA antibodies, which are referred to, as natural antibodies because they are induced without deliberate immunization, do not depend on antigenic exposure and contribute to the homeostasis and competence of the primary humoral immune system [4]. Natural IgG autoantibodies are present in the plasma of healthy individuals and, as a result, in pooled therapeutic IVIg preparations. They are considered key to the immunoregulatory effects of immune globulin in immune-mediated disorders [5]. Natural autoantibodies appear to be more polyreactive

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than immune antibodies and are frequently reactive with a wide spectrum of membrane-associated, intracellular, nuclear, and circulating self-antigens, including immunoglobulins' idiotypes [6–10].

Anti-idiotypic activity of IVIg is probably the most important mechanism of action of IVIg in the treatment of autoimmune diseases, and it results in short-term neutralization of autoantibodies. The presence of anti-idiotypes to autoantibodies within IVIg preparations has been confirmed by the inhibitory effect of IVIg on antiphospholipid antibodies, especially anticardiolipin (aCL), and lupus anticoagulant (LAC) [11–16]. Caccavo et al. [11] reported the inhibition of aCL binding to cardiolipin by F(ab')<sub>2</sub> fragment from IVIg in a dose-dependent manner. Galli et al. [12] demonstrated dose-dependent inhibition of LAC activity, using either IVIg or F(ab')<sub>2</sub> fragments from it. Partial neutralization of LAC activity was found in 10 of 11 patient sera following incubation with IVIg [13].

IVIg therapy has been successfully used as treatment for recurrent pregnancy loss associated with antiphospholipid antibodies, elevated circulating NK cells and embryotoxins, and abnormal NK cell activity as well as unexplained recurrent spontaneous abortions [3,6,17].

Elastin and collagen are the two main structural proteins of the body. Elastin is an unusual biological substance. It is one of the most hydrophobic polypeptides, the least soluble protein in the body and extremely durable, lasting the lifetime of the organism. Elastin is an essential mechanical component of many tissues including arterial wall, skin and lung. It could be considered a polymer of linear polypeptide chains (tropoelastin) stabilized by lysine-derived crosslinks, such as desmosine, isodesmosine, 5-dehydrolysylnorleucine, or lysylnorleucine. The soluble precursor of elastin has a lysine content of about 40 residues/1000 amino acid residues.

Autoantibodies to  $\alpha$ -elastin (elastin breakdown product) and tropoelastin (elastin precursor) are found in the serum of healthy human subjects. These physiological autoantibodies are assumed to be a part of a homeostatic mechanism, which clears, altered elastin structures via *in situ* destruction or via opsonization of the products of degradation. A marked increase in pathological anti- $\alpha$ -elastin autoantibodies was found in patients with autoimmune diseases – SLE, scleroderma and polyarteritis nodosa [18–20] and their role in the pathogenesis of autoimmune alterations have been suggested. The levels and pathogenic role of anti-elastin autoantibodies in RPL have not been objects of investigation.

Since the pooled therapeutic IVIg preparations consist of natural autoantibodies mainly, it could be suggested that similarly to the native sera, these preparations would be self-reactive to human elastin. Furthermore, the anti-idiotypes to anti-elastin autoantibodies of IVIg preparations would bind and inhibit the reactivity of anti-elastin autoantibodies in human sera under pre-incubation *in vitro*. The ability of IgG in IVIg preparations to bind both to elastin antigens and anti-elastin idiotypes by highly specific immune reactions could be an additional mechanism to explain the beneficial effect of IVIg in RPL.

The aim of this study was to investigate the serum levels of anti-elastin IgG autoantibodies in patients with RPL and use them in competitive inhibition studies for identification of anti-anti-elastin (anti-idiotypic) autoantibodies in different IVIg lots. Additionally, by one way analysis of variance (ANOVA) and multiple comparison test – Least Significant Difference (LSD method) we aimed to analyze the differences in IVIg-derived physiological anti-elastin autoantibodies and anti-elastin anti-idiotypes between different IVIg lots.

## 2. Materials and methods

### 2.1. Patients and controls

Serum samples were obtained with informed consent from 10 female patients with RPL and 10 normal non-pregnant women. The patients were selected on the basis of the following criteria: age (mean age 30, range 25–40 years), recurrent pregnancy loss which was defined as two or more consecutive pregnancy losses before 10 weeks' gestation, positive one or more of the following autoantibodies: anti-annexin V (ELISA, Orgentec), anti-prothrombin (ELISA, AESKU Laboratories, Wendelsheim, Germany), anticardiolipin, anti-beta2-glycoprotein I, anti-thyroglobulin, and/or anti-thyroid peroxidase (TPO) (ELISA, Clin Pro Int., USA). The blood samples were collected from 5 to 10 days after the last pregnancy loss. The non-pregnant control women (mean age 31, range 28–39) had normal reproductive histories of one or two live births and denied previous pregnancy losses.

### 2.2. IVIg lots

A total of 44 aliquots of different IVIg lots, IgG concentration 60 mg/ml, were kindly presented by ZLB Behring, Switzerland. The vials of the lots were numbered as follows: 001–020, 022, 101–121, 201, 202. The countries of origin for plasma pool were as follows: internal numbers 001–022 – USA, internal numbers 101–121 – European Union, internal numbers 201 and 202 – Switzerland.

### 2.3. Preparation of human aortic elastin

Human insoluble elastin was prepared from macro- and microscopic unaltered regions of thoracic aortas, obtained from 10 accident victims, 18–30 years old, using the method of Starcher and Gallione [21]. Amino acid analysis of the purified elastin showed quantitative similarity to the elastin, previously purified [21] and the lack of methionine suggested a low level of contamination. Soluble  $\alpha$ -elastin was obtained by the method of S.M. Partridge et al. [22].

### 2.4. ELISA for determination of anti-elastin IgG antibodies in IVIg lots

Samples of all 44 IVIg lots in nine IgG concentrations each (0.187  $\mu$ g/ml, 0.375  $\mu$ g/ml, 0.75  $\mu$ g/ml, 1.5  $\mu$ g/ml, 3.0  $\mu$ g/ml, 6.0  $\mu$ g/ml, 12.0  $\mu$ g/ml, 24  $\mu$ g/ml, and 48  $\mu$ g/ml) were tested

for antielastin IgG antibodies via direct homemade ELISA. A 96-well microtitre plate was coated with elastin by adding 100  $\mu$ l of a solution of  $\alpha$ -elastin (10  $\mu$ g/ml dissolved in 0.05 M carbonate buffer, pH 9.6) to each well and incubating for 2 h at 37 °C and overnight at 4 °C. Wells were washed with a solution of PBS, containing 0.05% Tween 20 (PBS-Tween) and then blocked by incubation for 1 h with 1% bovine serum albumin (BSA) in PBS-Tween. After washing with PBS-Tween, 100  $\mu$ l of IVIg lots, diluted from 48  $\mu$ g/ml to 0.187  $\mu$ g/ml in PBS-Tween were added. The plate was incubated for 1 h at 37 °C. Wells were then washed with PBS-Tween, incubated with a peroxidase-linked anti-human IgG (Sigma, diluted 1:5000 in 1% human serum albumin in PBS-Tween) and reacted with *o*-phenylenediamine as colorimetric substrate after washing. The reaction was terminated by 50  $\mu$ l 8 M H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 492 nm on automatic micro-ELISA plate reader.

For anti-elastin IgG antibody determination and statistical analysis, IVIg lots were tested in 7 groups of randomly chosen lots: Group A – lots 001, 002, 003, 114, 115, and 116; Group B – lots 109, 110, 111, 113, 117, and 118; Group C – lots 007, 011, 012, 013, 017, and 104; Group D – lots 016, 019, 020, 022, 119, and 120; Group E – lots 101, 102, 103, 105, 107, and 108; Group F – lots 004, 005, 006, 008, and 009; and Group G – lots 010, 014, 015, 018, 106, 112, 121, 201 and 202.

## 2.5. Pre-incubation of human sera with IVIg lots

For idiotype-anti-idiotype interactions between anti-elastin autoantibodies of the human sera and IVIg-derived anti-idiotypic antibodies (anti-anti-elastin IgG antibody) in the lots, samples of the tested human sera, diluted 1:10 in PBS-Tween, were preincubated with increasing concentrations (0.187  $\mu$ g/ml, 0.375  $\mu$ g/ml, 0.75  $\mu$ g/ml, 1.5  $\mu$ g/ml, 3  $\mu$ g/ml, 6  $\mu$ g/ml) of the lots for 2 h at 37 °C and overnight at 4 °C.

## 2.6. Competitive ELISA for determination of anti-elastin IgG autoantibodies in human sera

Anti-elastin IgG autoantibodies in the human sera were tested without preincubation (patients and controls) and after preincubation (patients) with different IgG concentrations of the lots using the ELISA method described. Briefly, 96-well microtitre plates coated with  $\alpha$ -elastin and blocked with 1% BSA in PBS-Tween were used. After washing with PBS-Tween, 100  $\mu$ l of preincubated and non-preincubated sera (1:10 in PBS-Tween) were added. The plates were incubated for 1 h at 37 °C. Wells were washed with PBS-Tween, incubated with a peroxidase-linked anti-human IgG (Sigma, diluted 1:5000 in 1% human serum albumin in PBS-Tween) and reacted with *o*-phenylenediamine after washing. The reaction was terminated by 50  $\mu$ l 8 M H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 492 nm.

The inhibition of serum anti-elastin IgG antibody by preincubation with IVIg lots was tested in 9 Inhibition Groups (IG): IG-A – Serum 1 preincubated with 6 lots, IG-A-1 – Serum 2

preincubated with the same 6 lots (as in IG-A); IG-B – Serum 1 preincubated with 6 lots; IG-C – Serum 3 preincubated with 5 lots; IG-D – Serum 4 preincubated with 5 lots, IG-D-1 – Serum 5 preincubated with 5 lots (the same as in IG-IV), IG-E – Serum 6 preincubated with 6 lots, IG-F – Serum 7 preincubated with 7 lots, IG-G – Serum 8 preincubated with 2 lots, IG-H – Serum 9 preincubated with 2 lots.

Modulation (in %) of serum anti-elastin autoantibody after preincubation with IVIg was established according to the following calculations:

$$\begin{aligned} \text{O.D of serum anti-elastin IgG antibody (A)} &= 100\% \\ \text{O.D. of serum anti-elastin IgG antibody after preincubation (B)} &= X\% \\ X\% &= (B \times 100): A \\ X\% - 100\% &= Y\% \end{aligned}$$

Y is the percentage of change – increase (when is a positive number) or decrease (when is negative) of serum anti-elastin reactivity after pre-incubation with IVIg.

## 2.7. Statistical analysis

Differences in anti-elastin IgG autoantibodies between the patients and controls as well as between the lots in each group were analyzed for statistical significance ( $P < 0.05$ ) with one-way analysis of variance (ANOVA). When the overall comparison of the lots was significant we applied multiple comparison test – Least Significant Difference (LSD method) to determine which individual lots in the group differ. By ANOVA and LSD method we compared anti-elastin antibody of lots for the last four concentrations of IgG (6.0  $\mu$ g/ml, 12.0  $\mu$ g/ml, 24  $\mu$ g/ml, and 48  $\mu$ g/ml). This was indicated by a preliminary analysis of all 9 concentrations, which could not identify the differences because of the similarity of the low concentrations results.

Similarly, differences between the percentages of modulation of serum anti-elastin antibody in each inhibition group were examined by one-way analysis of variance (ANOVA). LSD method for the last four pre-incubation concentrations of lots' IgG (0.75  $\mu$ g/ml, 1.5  $\mu$ g/ml, 3  $\mu$ g/ml, and 6  $\mu$ g/ml) was applied when the overall comparison of the lots was significant. The statistical package used was SPSS v.11.5.

## 3. Results

### 3.1. Anti-elastin IgG autoantibodies in patients and IVIg lots

The mean level of anti-elastin IgG autoantibodies in the study group was increased compared with the controls (patients – mean 0.453, SD 0.142; controls – 0.267, SD 0.079). The difference in anti-elastin IgG autoantibodies between patients and controls was significant –  $F = 1.321$ ,  $P = 0.005$ .

Anti-elastin IgG autoantibodies were identified in all tested 44 lots and their levels were similarly dose-dependent. On

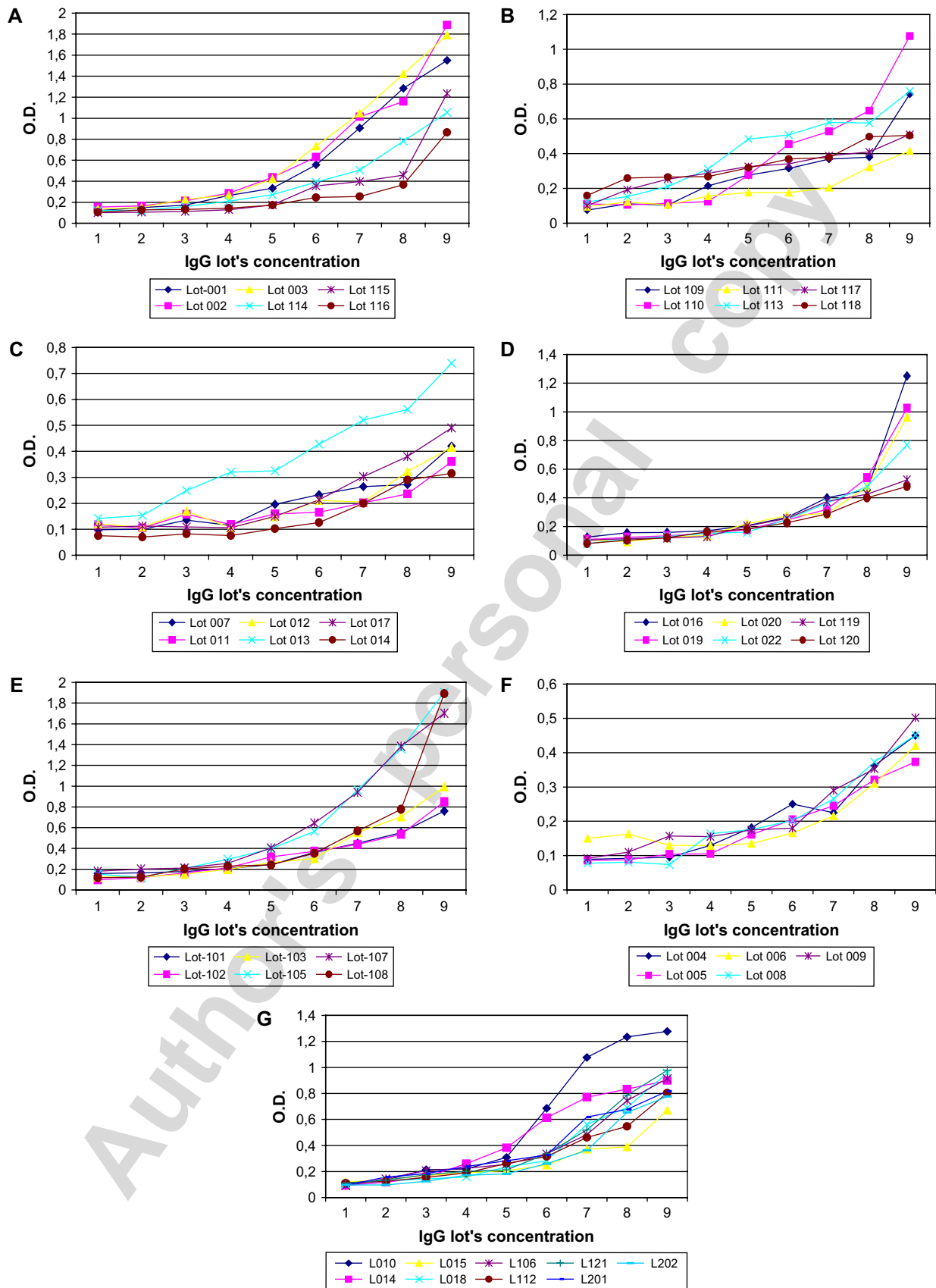


Fig. 1. A–G. Representative data of anti-elastic IgG autoantibodies in IVIg lots detected by ELISA. The lots were tested in 7 groups (A–G) of randomly chosen lots. Increasing optical density values (vertical axis) were observed with increasing concentrations (from 0.187  $\mu\text{g/ml}$  to 48  $\mu\text{g/ml}$ ) of IgG in IVIg lots (horizontal axis).

Table 1

One way analysis of variance (ANOVA) of the differences between anti-elastin IgG autoantibodies in IVIg lots within each tested group of lots

Group		Sum of Squares	Mean Square	F	P value
Group A	Between groups	2.274	.455	2.667	.057
	Within Groups	3.070	.171		
Group B	Between groups	.407	.081	<b>3.260*</b>	<b>.029*</b>
	Within Groups	.449	.025		
Group C	Between groups	.297	.059	<b>7.707*</b>	<b>.003*</b>
	Within Groups	.188	.010		
Group D	Between groups	.161	.032	.385	.853
	Within Groups	1.502	.083		
Group E	Between groups	1.832	.366	1.878	.148
	Within Groups	3.512	.195		
Group F	Between groups	.009	.002	.196	.937
	Within Groups	.178	.012		
Group G	Between groups	1.120	.140	<b>2.582*</b>	<b>.031*</b>
	Within Groups	1.464	.054		

\*Differences between lots are statistically significant.

Fig. 1A–G, the results from the determination of anti-elastin IgG autoantibodies in the seven tested groups of lots are presented. Lot's IgG concentrations (X-axis) are numbered as follows: 1 – 0.187 µg/ml, 2 – 0.375 µg/ml, 3 – 0.75 µg/ml, 4 – 1.5 µg/ml, 5 – 3.0 µg/ml, 6 – 6.0 µg/ml, 7 – 12.0 µg/ml, 8 – 24 µg/ml, and 9 – 48 µg/ml.

### 3.2. Statistical analysis

Results from examination of differences between anti-elastin autoantibodies of lots in each group by one-way analysis of variance (ANOVA) are presented on Table 1. Overall comparison of the lots was significant in Groups B, C and G ( $F > 3.0$ ,  $P < 0.05$ ).

Results from LSD method applied for groups with significant overall comparison (Groups B, C and G) are presented on Table 2 (significant differences only are shown). In Group B, significant differences of anti-elastin IgG autoantibodies were established between lot 110 and lots 111, 117, and 118 as well as between lot 111 and lot 113. In Group C, significant differences were established between lot 013 and all the rest – 007, 011, 012, 017, and 104. In Group G, significant differences were established between lot 010 and all the rest except lot 04, as well as between lot 014 and 015 (Table 2).

### 3.3. Inhibition of serum anti-elastin IgG autoantibodies by pre-incubation with IVIg lots

Testing of anti-elastin IgG autoantibodies in patients' sera before and after pre-incubation with IVIg lots showed dose-dependent modulation of the levels of these antibodies most probably due to idiotype-anti-idiotype interactions. Results from inhibitions of serum anti-elastin IgG autoantibodies by pre-incubation of the sera with the lots are presented on Fig. 2A–H. Results are presented in percentages according to the calculations described. Each Inhibition Group (IG) consists of one serum pre-incubated with different lots. In cases of IG-A (Fig. 2A) and IG-A-1 (Fig. 2A1) and IG-D (Fig. 2D)

and IG-D-1 (Fig. 2D1) we compare two different sera pre-incubated with the same lots.

### 3.4. Statistical analysis

The examination of differences between the modulation effects of lots on serum anti-elastin autoantibodies in each Inhibition Group by overall comparison of the lots (ANOVA) established significance ( $F > 3.0$ ,  $P < 0.05$ ) in Inhibition Groups A-1 ( $F = 3.667$ ,  $P = 0.018$ ) and C ( $F = 8.626$ ,  $P = 0.001$ ).

When the overall comparison of the lots was significant (IG-A-1 and C) we applied multiple comparison (LSD

Table 2

Multiple comparison (LSD method) of anti-elastin IgG autoantibodies in IVIg lots from Groups B, C, and G

Comparison		Mean Difference (I–J)	P value
Lot (I)	Lot (J)		
Group B			
Lot 110	Lot 111	.39700*	.002
	Lot 117	.26425*	.029
	Lot 118	.23928*	.046
Lot 111	Lot 110	–.39700*	.002
	Lot 113	–.32600*	.009
Group C			
Lot 013	Lot 007	.26475*	.002
	Lot 011	.32125*	.000
	Lot 012	.27375*	.001
	Lot 017	.21550*	.008
	Lot 104	.32925*	.000
Group G			
Lot 010	Lot 015	.64800*	.001
	Lot 018	.44475*	.012
	Lot 106	.44850*	.011
	Lot 112	.53625*	.003
	Lot 121	.41325*	.018
	Lot 201	.45850*	.010
	Lot 202	.55450*	.002
Lot 014	Lot 015	.35875*	.038

\*The mean difference is significant at the .05 level.



method) to determine which individual lots differ. Results (significant differences only) are presented on Table 3. In IG-A-1, significant differences in inhibitions were established between lot 109 and lots 110 and 111 as well as between lot 113 and lots 110, 111, 117, and 118. In IG-C, significant differences in inhibitions were established between lot 004 and lots 006, 008, and 009 as well as between lot 005 and lots 006, 008, and 009. The examination of differences (ANOVA) in IG-E did not show significance but multiple comparison (LSD method) determined that some individual lots differ. In IG-E, significant differences in inhibitions were established between lot 019 and lots 022, 119, and 120 as well as between lot 020 and lot 119.

#### 4. Discussion

The role of the extracellular matrix protein elastin in RPL is poorly understood. Jerzak et al. found significantly increased T cell adhesion to collagen IV, fibronectin and elastin in women with the history of RPL and low-dose IVIg normalized this effect [17].

The role of anti-elastin autoantibodies in RPL has not been an object of previous studies. Significant increase was established in our patients compared to the controls, however this finding needs further confirmation in larger study groups. To our knowledge, this is the first study on IVIg-derived anti-elastin autoantibodies. In all lots anti-elastin IgG autoantibodies were

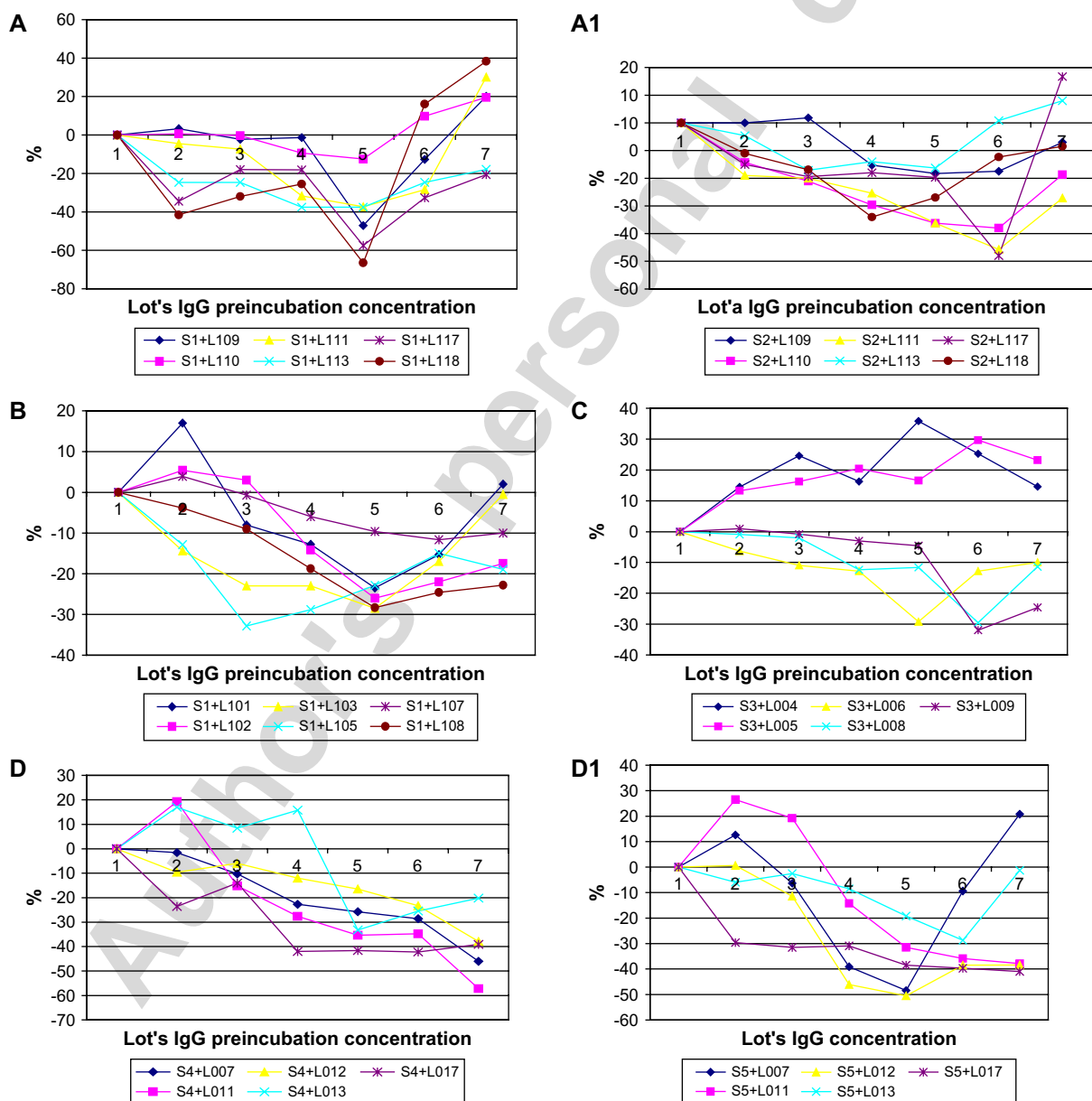


Fig. 2. A–H. Inhibition of patients' anti-elastin IgG activity by IgG in IVIg lots. Increasing concentrations of lots' IgG were pre-incubated with fixed dilutions of patients' sera and anti-elastin IgG autoantibodies were then assessed. The results for each Inhibition Group are presented in separate figures (A–H). The legends show the number of the pre-incubated serum (S) and lots' numbers (L). The vertical axis indicates the modulation (in %) of anti-elastin IgG autoantibodies according to the calculation described. The horizontal axis indicates the pre-incubation concentration of IgG in IVIg (from 0.187  $\mu\text{g/ml}$  to 6.0  $\mu\text{g/ml}$ ).

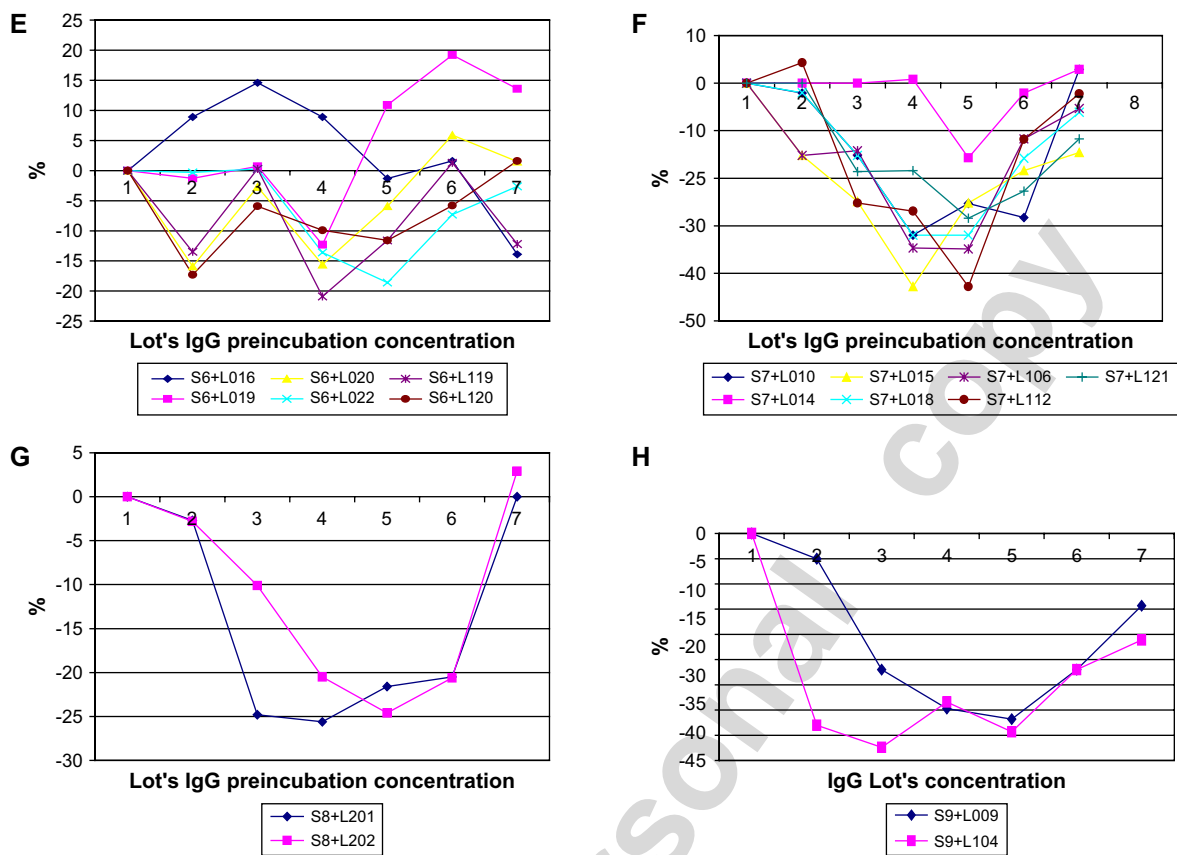


Fig. 2 (continued).

determined and their levels were dose-dependent. This finding is in accordance with previous studies on physiological anti-elastin autoantibodies established in sera of healthy subjects [23,24]. Wei et al. established that monoclonal and polyclonal anti-human aorta elastin antibodies stained elastic fibers on tissue sections, suggesting that the epitopes recognized are available on the native fibers for reactions with the antibodies [25]. Physiological anti-elastin autoantibodies together with anti-tropoelastin autoantibodies and elastin-derived peptides are markers of elastin degradation and synthesis. Anti-elastin antibodies may play a physiological role in the remodeling of injured or senescing elastic structures by binding and clearance of elastin degradation products. It seems that IVIg preparations contain the physiological anti-elastin antibodies existing in the sera of healthy plasma donors since all the lots showed specific dose-dependent reaction with the antigen – human aortic  $\alpha$ -elastin.

LSD analysis of the differences between the levels of anti-elastin IgG autoantibodies in groups of lots showed that these antibodies were significantly different in five lots compared to the rest number of lots (Table 2). If we assume that there were three main geographical areas of origin of plasma pools – USA, EU and Switzerland, we could see from the analysis, that each one of the different lots shows difference in anti-elastin antibodies not only compared to the lots from the other areas but also to lots from the same area. We could conclude that the levels of anti-elastin antibodies in IVIg lots are

depending rather on their individual levels in the plasma of the donors but not on the geographical area.

Natural IgG autoantibodies are reactive not only with circulating self antigens but also with the idiotypes of immunoglobulins. The idiotypic represents the highly variable antigen-binding

Table 3  
Multiple comparison (LSD method) of lots in Inhibition Groups A-1, C and E

Comparison		Mean Difference (I–J)	P value
Lot (I)	Lot (J)		
Group IA			
109	110	.08025*	.046
	111	.08675*	.032
110	113	–.12550*	.004
	111	–.13200*	.002
113	117	.10050*	.015
	118	.07825*	.051
Group III			
004	006	.16250*	.006
	008	.21275*	.001
	009	.21200*	.001
005	006	.15300*	.009
	008	.20325*	.001
	009	.20250*	.001
Group V			
019	022	.05550*	.014
	119	.05675*	.012
	120	.04325*	.047

\*The mean difference is significant at the 0.05 level.



site of an antibody and is itself immunogenic. During the generation of an antibody-mediated immune response, an individual will develop antibodies to the antigen as well as anti-idiotypic antibodies, whose immunogenic binding site (idiotype) mimics the antigen. Several lines of evidence suggest that IVIg contain anti-idiotypes against a variety of autoantibodies from patients with a variety of autoimmune diseases: anti-thyroglobulin antibodies [26], regulatory anti-Sm idiotype [27], anti-DNA and antiphospholipid auto-Abs [28], anti-intrinsic factor antibodies [29], etc. For the first time, we determined anti-anti-elastin idiotypes in IVIg by competitive inhibition studies against human serum anti-elastin antibodies. We observed that IVIg inhibited the binding of anti-elastin IgG antibodies to their antigen — human aortic  $\alpha$ -elastin. The inhibitory effect of IVIg was dependent on interactions between the variable regions of IVIg and variable regions of anti-elastin autoantibodies. Preincubation of sera with IVIg resulted in the specific retention of autoantibody activity, demonstrating that naturally occurring anti-idiotypic antibodies reactive with anti-elastin antibodies are detectable in IVIg lots and are predominantly of the IgG isotype.

All lots (except lot 004 and 005) showed similar dose-dependent inhibition of anti-elastin activity by anti-elastin anti-idiotypes in IVIg with maximal inhibition occurring at a specific molar ratio between serum IgG and IVIg and a prozone phenomenon.

LSD analysis of the differences showed that the inhibition caused by seven of the lots was significantly different compared to the inhibition of the same serum caused by the other lots in the Inhibition Group. Since we compared one serum preincubated with different lots, it seems that the difference is due to the level of physiological anti-anti-elastin autoantibodies in the lots (anti-idiotypes) but not to the anti-elastin autoantibodies in the serum (idiotype). We could conclude that the relative content in anti-anti-elastin idiotypes against an anti-elastin autoantibodies may differ between IVIg lots.

When the calculated percentage of change ( $Y$ ) was a positive number (as in lots 004 and 005, Fig. 2C) we could speculate that in these lots the level of anti-anti-elastin autoantibodies was very low or missing in the tested concentrations, therefore, after preincubation with the serum, serum anti-elastin antibodies not only did not decrease but increase because of anti-elastin antibodies added from the lot. Two incubation samples — S1 + L118 and S2 + L117 (Fig. 2A and A-1), showed a jump from 60% (S1) and 50% (S2) inhibition to 20% enhancement following preincubation. These were the highest inhibitions and enhancements achieved. We suggest that this phenomenon is due to: 1) high lot level of anti-anti-elastin idiotypes, causing maximal anti-elastin inhibition; 2) prozone effect with the higher lot's IgG concentrations due to anti-anti-elastin idiotype excess resulting in decreased inhibition; and 3) additional reaction of anti-elastin antibodies from the lots in the highest lot's IgG concentrations when the inhibition was not more effective.

In conclusion, anti-elastin autoantibodies were increased in patients with RPL. Further investigations are necessary to reveal the clinical relevance of these autoantibodies for the pregnancy loss. Physiological anti-elastin and anti-anti-elastin autoantibodies were identified in different IVIg lots. The presence in IVIg of

anti-idiotypes against anti-elastin autoantibodies from patient's sera may provide an additional mechanism for the beneficial effect of IVIg in RPL and supports the concept of a functional idiotypic network regulating autoimmune responses in humans.

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