

Bovine serum albumin immunization: a new risk of allergy during protocols for *in vitro* fertilization

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Three patients having undergone protocols for *in vitro* fertilization (IVF) showed an allergic reaction suggesting seric disease 6 to 10 days after follicle aspiration. The responsibility of bovine serum albumin, present in the follicle-rinsing fluid and in the medium used for embryo culture was suspected. An immunological study was carried out, using two groups for comparison. Group 1: 7 controls. Group 2: 11 women having undergone IVF protocols without incident. Anti-BSA antibodies were present in low quantities in one or two subclasses of IgG and IgA in Group 1, and in several subclasses at higher levels in nine of 11 subjects from Group 2. The IgG₄ response predominated 7 times out of 9. The antibody content gradually fell after the IVF attempt. An immune response predominated in the IgG₁ in two patients. Skin test for the two fluids and BSA were positive only in the three patients. These results show the frequency of immunization to BSA induced through the ovarian and peritoneal pathways. Seric disease corresponds to a rise in IgG₁. The association of a IgE-dependent sensitization is suspected. The authors suggest the possibility of an interference of these immune processes with implantation and propose the elimination of heterologous proteins from fluids. They also suggest that LH-RH analogues facilitate immune responses.

Key words: IVF-BSA-immunization; seric disease; sub-class IgG antibodies.

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Since 1978, *in vitro* fertilization techniques (IVF) have been widely developed (3-9). Preceded by hormone treatment, they include two phases: the aspiration of mature ovarian follicles for the recovery of oocytes (A), then, after 48 h in a medium in the presence of spermatozooids, the reimplantation of embryos in the uterine cavity (R). The few reactions of an allergic type which have been observed during the hormone treatment have been attributed to the histamine-releasing effect of LH-RH analogues (1). The possibility of drug allergy to polypeptide hormones cannot be ruled out because immunization may be due to the repetition of treatments (7). A first case of an allergic reaction 8 days after A-R, could be

attributed to a bovine serum albumin (BSA)-induced seric disease (14). This protein is present in the fluid used to rinse the follicles and in the medium for embryo culture. Later, two other cases were observed, and led to a search for the appearance of an immune response to this heterologous protein in women who underwent these protocols without incident. The study shows the high incidence of a humoral immune response, the profile of which differs in women with no clinical response (predominance of specific IgG₄) from that in women with a seric disease (predominance of IgG₁). These results raise the question of a possible interference of these immune processes with the nidation.

MATERIAL AND METHODS

All subjects gave their informed consent.

Group A: Seven subjects from 16 to 54 years old (6 women and 1 man): three have no atopy and four are atopic (allergic asthma or hay fever). A male was included in controls as he was atopic with a food allergy to beef attested by clinical history, a positive prick-test and a class 3 RAST®.

Group B: 11 women from 27 to 39 years old having undergone a number of attempts at IVF: one attempt (6), two (1), four (3), seven (1). Three of them sought consultation for allergic skin reaction during the hormone treatment. No incidence was observed with the other eight.

Group C: Three patients from 31 to 35 years old. B.M.C developed severe urticaria and a temperature 8 days after A on the second attempt. M.E. had generalized urticaria and facial Quincke's oedema 10 days after AR on the first attempt. B.M. had urticaria 7 days after AR on the third attempt, and 6 months later, urticaria 6 days after A on the fourth attempt.

The patients in Groups B and C were studied with various time lapses after protocol (from 8 days to 9 months). Two patients from Group B were comparatively studied before and 8 days after AR. Two patients from Group C gave two successive blood samples after AR.

The search for atopy was carried out by questionnaire, prick tests with pollens, house dust mites and cat epithelia (Stallergen extracts - Fresnes France) and a Phadiatop®. In three patients from Group B, the search for a drug allergy to polypeptidic hormones or to HMG was carried out by prick tests, intradermal tests (ID-T), and human basophil degranulation test (HBDT) according to techniques and criteria of interpretation already published (8).

The follicle-rinsing fluid: Menezo/IMV/IN-RA (ref. UA024) and the *in vitro* medium: B2 Menezo (7), contain BSA: respectively 4 gm/l and 10 gm/l (11). The BSA tested is the SIG-MA No. A 7638 crystallized BSA in solution of 1 mg/ml. The two fluids were tested pure in pricks, diluted to 1/10 in ID.T.

Groups B and C were tested by pricks, then ID.T. Group A was tested only by pricks.

Immunometric enzyme immuno tests were performed for the determination of anti BSA IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, IgA₂ and IgE in experimental and control sera.

Reagents and sera

Control sera were obtained from 12 human volunteers who were non-atopic, who did not present any food allergy, and who had a negative RAST® to BSA. BSA (fraction V) was purchased from I.B.F. and purified by gel filtration on G50 Sephadex. Human serum albumin (HSA) was obtained from the Centre National de Transfusion Sanguine. Monoclonal anti-human IgE antibodies, labelled with acetylcholinesterase (AChE) were obtained from the Laboratoire des Stallergènes. Swine anti-murine IgG antibodies, labelled with acetylcholinesterase were kindly provided by the Laboratoire d'Etudes Radioimmunologiques, Centre d'Etudes Nucléaires de Sacaly. Murine monoclonal anti-human IgG and IgA subclass antibodies were purchased from Unipath for anti-IgG₁, IgG₃, IgG₄; from Biomakor for anti-IgG₂; from Sera-Lab for anti-IgA₁, anti-IgA₂.

Solid phase enzyme immunoassay (EIA) was performed by using specialized Titertek microtitration equipment including an automatic plate washer (Microplate Washer 120), an automatic dispenser (Autodrop) and a spectrophotometer (Multiskan MCC° set at 414 nm, all from Flow Laboratories). Microtiter plates were 96 F/immuno plates I with certificate from NUNC.

Procedures

Ninety-six well microtiter plates were coated with purified BSA (5 mg/l in 0.025 M EDTA pH 9.3 buffer) and the indirect ELISA was performed according to Campbell et al. (2). After incubation with anti-Ig-AChE, the AChE activity was measured by the colorimetric method of Ellman (6). Dilutions of AChE-labelled antibodies were determined so that the enzymatic tracer was used at a concentration of 1 Ellman unit/ml.

EIA of anti BSA IgG and IgA subclasses was performed as follows:

- 1) 50 μ l of two-fold dilution (from 1/40) of each serum, are placed in six wells,
- 2) washing,
- 3) 50 μ l of murine monoclonal anti-human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, IgA₂ antibodies at convenient dilutions in EIA buffer are placed in the different wells. Contact is allowed for 4 h at 20°C,
- 4) washing,
- 5) 50 μ l of swine anti-murine IgG antibodies conjugated to AChE are added. Contact is allowed for 18 h at 20°C.
- 6) washing,
- 7) colorimetric measurements.

For each experiment the colorimetric measurement carried out on the control sera at the first dilution (e.g. 1/1 for IgE determinations, 1/40 for IgG and IgA sub-class determinations) that were not different from the blank determinations on wells containing only EIA buffer instead of serum, were expressed as mean $\pm$ SD. Determinations on patients' sera at different dilutions were considered positive when the optical density measured was $>$ mean + 3 SD. Results are thus expressed for each serum as the last positive dilution.

RESULTS

Group A: Five controls showed a low level of anti-BSA antibodies in one or two IgG and IgA subclasses, with a more frequent response in the IgA₁. No difference was observed between the three non-atopics and the three atopics. The subject allergic to beef had higher levels of IgG₄:

1/640°, and of IgG₁, and IgG₂. There were no IgE antibodies (Table 1). All tests for anti-HSA antibodies were negative. Prick tests with two fluids and BSA were negative.

Group B: Of 11 patients, one woman was atopic (1). In three patients who suffered from urticaria during the hormone treatment, a probable drug allergy was diagnosed by skin tests with triptorelin (Case No. 1), buserelin (Case No. 9) HMG (Case No. 6). Case No. 11 had a previous history of intolerance to glafenine.

Nine patients of 11 had anti-BSA antibodies in the various IgG and IgA subclasses: G₁: 9, G₂: 10, G₃: 7, G₄ 8, A₁: 9, A₂: 3. Seven of nine have a principal response in the IgG₄ (Table 3). The comparative study of sera removed before and 8 days after AR (Cases 1 and 2) shows a noticeable increase in IgG₄, IgG₁, IgG₂ antibodies and the appearance of IgG₃ and IgA₂ antibodies. The lowest levels are found in patients 10 and 11, whose serum was studied long after AR. Two patients (7 and 8) have no anti-BSA antibodies. There are no IgE antibodies. Tests for anti-HSA antibodies remain negative. Prick tests and ID-R are negative with the two fluids and with BSA.

Group C: Anti-BSA antibodies are present in the various subclasses, with a low level of IgG₄ antibodies which disappears at the second sampling, and a high level of IgG₁ antibodies in one patient. The third has a higher level of IgG₄. The global response weakens in the serum removed a long time after the incident. There are no IgE antibodies. No cross-reactivity with HSA is shown. Prick tests are positive with fluid 2 in Cases 1 and 2 and with fluid 1 in Case 1.

Table 1
Levels of anti-BSA antibodies in the IgG and IgA subclasses in 7 controls

Case	Atopy	Food allergy	G ₁	G ₂	G ₃	G ₄	A ₁	A ₂	E
1	-	-	-	-	-	-	40	-	-
2	-	-	-	40	-	-	40	-	-
3	-	-	-	-	-	-	40	-	-
4	+	-	-	-	40	-	-	-	-
5	+	-	-	-	-	-	-	-	-
6	+	-	-	40	-	-	40	-	-
7	+	+ beef.	-80	80	-	640	-	-	-

Table 2

Anti-BSA antibodies in 11 women having undergone IVF protocols without incident

Cases	Age	A	R	Date of blood sample	Anti BSA-antibodies					
					G ₁	G ₂	G ₃	G ₄	A ₁	A ²
1	32	1	1	before + 8 d	320 2560	80 1280	- 640	160 640	40 640	- 320
2	34	4	4	before + 8 d	640 1280	80 1280	- 640	640 40.960	40 640	- -
3	36	2	1	+ 8 d	320	320	160	20.480	160	40
4	34	4	4	+ 8 d	1280	1280	160	10.240	160	-
5	39	7	3	+ 8 d	320	160	-	1280	160	-
6	30	4	4	+ 8 d	160	80	-	2560	40	-
7	35	1	1	+ 8 d	-	40	-	-	-	-
8	34	1	1	+ 8 d	-	-	40	-	-	-
9	31	1	1	+ 15 d	80	320	40	1280	160	40
10	38	1	1	+ 4 mo	320	40	-	160	40	-
11	27	1	1	+ 9 mo	80	40	40	-	40	-

A: follicle aspiration; R: embryo reimplantation; Case. 1: Penicillin and triptorelin drug allergy; Atopy 6: HMG D. A.; 9: Buserelin D. A.; 11: Intolerance to glafenin and paracetamol.

ID-T are positive with fluid 2 and with BSA in three cases (Table 3). HBDT is positive with washed basophils, to BSA and to fluid 1, in Case 1 only.

DISCUSSION

Three patients showed delayed allergic reactions 6 to 10 days after oocyte aspiration, with or without subsequent reimplantation. After the fact it was logical to suspect a mild seric disease

related to BSA, a heterologous protein used in high concentrations in rinsing fluid (4 g/l) and embryoculture medium, in our center, as in other clinics. Aspiration with follicle-rinsing brings about a close contact with the ovarian tissue and inevitably with the peritoneum. Upon reimplantation, traces remain around the embryo, in contact with the uterine mucosa. Thus there is a possibility of stimulation of the local immunological systems, GALT and MALT, associated with the female genital

Table 3

Skin tests and levels of anti-BSA antibodies in three patients with post-IVF seric disease

Case	A	R	Skin tests			Delay after AR	G ₁	G ₂	G ₃	G ₄	A ₁	A ₂	E
			Fluid 1	Fluid 2	BSA								
12	2	1	+ P	+ PI	+ I	+ 1 mo	10.240	160	80	80	80	-	-
						+ 6 mo	640	40	40	-	80	40	-
13	1	1	-	+ I	+ I	+ 2 d	640	80	320	160	640	-	-
						+ 1 mo	320	40	160	-	40	-	-
14	4	4	-	+ PI	+ PI		320	160	80	2560	80	-	-

P: prick; I: intradermal test.

tracts. The obstetrician used from 10 to 20 cc of fluid 1 to rinse the follicles, which represents 40 to 80 mg of BSA. Upon reimplantation, the amount of BSA was probably below 1 mg.

The possibility of immunization and sensitization to BSA is well known in man since it is a food antigen present in milk (10). Heat, however, readily transforms BSA. It has been shown that the immune response initiated orally in animals is physiologically weak, even non-existent (10-15). This explains why anti-BSA IgG content is low or non-existent in healthy humans, and why BSA is considered as a minor allergen in cases of milk allergy (13-21). The results of the controls confirm this. The antibody content is no more than 1/40, preferentially situated in the IgA₁. In the subject with beef allergy, the specific IgG₄ content is high (1/640°), but there are no specific IgE, though the RAST is very positive to beef extract.

Our findings are consistent with the hypothesis that BSA immunization is related to IVF. This is supported by two arguments. First, in Group 2 (protocol IVF) nine patients of 11 have a higher level of antibodies in several IgG subclasses than in Group 1. Second, in subjects 1 and 2, a very noticeable increase in these levels is observed in the serum tested 8 days after AR, compared with the serum before AR. As time passes, a low level is observed (Cases 10 and 11).

It is striking to note the large IgG₄ antibody content of nine subjects, considering that IgG₄ account for only 4% of all IgG. The response orients itself electively towards IgG₄. These antibodies are frequently observed in studies of food allergy. An allergic antibody activity of STS IgG type has been postulated in certain cases (16). However, these antibodies frequently coexist with specific IgE and it is not certain that the clinical manifestations can be attributed to them (12). Further, in the clinical treatment of food allergy in children, a drop in specific IgE and a high residual level of IgG₄ antibodies is often observed. This may justify attributing them a protective role, especially as they cannot activate the complement. They could be considered harmless from an allergic point of view, at least in this study, as no systemic allergic reaction was observed in Group 2.

In the three patients (Group C), the profile of the humoral response differs from that of Group B on two counts: firstly, the IgG₄ response is weak and transitory, disappearing shortly after the AR. Secondly, the response is predominant in the IgG₁. This subclass can activate the complement, and a relationship between seric disease and these IgG₁ antibodies can be postulated. The positivity of the skin tests to fluid 2 at 1/10 (corresponding to 1 mg/ml of BSA) corresponds to the positivity of the BSA itself observed at the same concentration. It is of an immediate type and is not accompanied by semi-delayed reactions. Thus, it is highly unlikely that IgG₁ are involved in skin reactivity. Furthermore, the nine patients from Group 2 have specific IgG₁ and their skin tests are negative. This reactivity may reveal a slight mast cell sensitization by specific IgE. In case 1, the degranulation of the washed basophils in the presence of BSA confirms the possibility of a slight IgE-dependent sensitization with no observable seric antibodies. The negative results of the ELISA technique could also be due to a competitive effect between the IgE antibodies and the high quantity of IgG antibodies. Recent reports confirm this isolated appearance of BSA-specific IgG, without specific IgE (4).

Groups B and C (14 patients) showed no anti-HSA antibody despite the 80% homology that exists between the HSA and BSA molecules. This is important, as at least one case of anaphylaxis to HSA has been described (18).

What stimulates this immune response to BSA? Taking into account the quantities of BSA present at aspiration and reimplantation, it would seem that aspiration triggers the immune response. Further, we have observed a reaction after A without R, two times out of four.

There is another argument: since early 1989 the technique has been changed. Thanks to improved endovaginal ultrasonographic scanning, follicle-rinsing has become unnecessary. No new case (out of > 200 A) has been observed. Rinsing had been used for 2 years and, *a posteriori*, we evaluate the prevalence of delayed allergic reactions at 1%, although Cistero (4) suggests a higher figure: 9%. In our center allergic incidents appeared in 1987 in connec-

tion with the prescription of LH-RH agonists. Although this clinical observation remains isolated, so far, it may be that LH-RH agonists encouraged BSA immunization either by interfering with the immune cells or because they are self-sensitizing, placing the organism in a state of immunological hyperactivity. Recent work has demonstrated the immunomodulating role of oestradiol and progesterone (19–20).

However, the presence of traces of BSA around the embryo may not be harmless. Indeed, the uterine mucosa is rich in mast cells, and various aspects of degranulation have been observed (5–17). Furthermore, it is capable of local immune response (22). One possible hypothesis is that prior immunization to BSA could induce a local reaction of the uterine mucosa, preventing implantation. Among the patients under investigation, reimplantation was an immediate failure in the three patients from Group C and in eight of 11 from Group B. In this group cases 1 and 4 had a miscarriage after 1 and 4 months, respectively. Only one reimplantation has resulted in a pregnancy, Case 8, who was one of the two women with no immune response to BSA.

Conclusion

Any new medical technique involves a risk of allergy. The injection of a heterologous protein is not a negligible factor, especially in protocols for IVF, as such an injection is repeated several times. The study of 11 patients undergoing IVF without incident allows the high frequency of immunization to be observed. It is noticeable that when an IgG₄ antibody response predominates, BSA appears well tolerated, whereas if it predominates in the IgG₁ there is a clinical reaction. Thus it can justifiably be suggested that specific IgG₄ play a protective role by competition with the other IgG sub-groups capable of activating the complement. The coexistence of IgE-dependent sensitization can be suspected because of the immediate positivity of skin tests in patients having suffered from seric disease related to IgG₁ antibodies. It can be suggested that, at least in those patients immunized to BSA, the reimplantation

of the embryo could fail, because of a possible reaction of sensitized mast cells in the uterine mucosa to the traces of BSA found around the embryo. A ban should be placed on all heterologous proteins in the fluid used to recover oocytes, as they are the main suspects in the immunization process. However, keeping a heterologous protein such as BSA in the culture medium may not be advisable either, since some patients could be immunized beforehand through food. The use of human serum albumin, patient serum, or serum, or serum-free medias is possible and could avoid the problem (7).

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