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Active or passive immunization in unexplained recurrent miscarriage

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Abstract

Controversy exists as to whether active immunotherapy with allogeneic lymphocyte transfusions (ALT) or passive immunotherapy with intravenous immunoglobulin (IvIg) improve the chance of live birth in women with unexplained recurrent miscarriages (RM). Meta-analyses of the placebo-controlled trials carried out as Cochrane reviews have concluded than none of the different forms of immunotherapy has proved effective in the total RM population. However, the included trials have generally been small and very heterogenous with respect to the clinical histories of patients and the immunization protocols. Thus, other meta-analyses which have looked at the efficacy in subgroups of RM patients have reported that ALT and IvIg may be effective in women with primary RM and secondary RM, respectively. In RM clinics in Denmark, ALT with donor lymphocytes or IvIg immunotherapy have been tested in several placebo-controlled trials since 1986 in which greater doses than used in other trials have been administered, and both treatments are now used for routine therapy. Our results have convinced us that using the correct immunization protocols on the right subsets of RM patients is effective, but we admit that new placebo-controlled trials focusing on subsets of RM patients are now urgently needed. Furthermore, treated patients should be extensively monitored for changes in a series of immune parameters that may predict pregnancy success and be of importance for our understanding of the mechanisms of action of immunotherapy in RM.

Keywords: Abortion; Immunotherapy; Intravenous immunoglobulin; Recurrent miscarriage; Recurrent spontaneous abortion

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1. Introduction

A significant proportion of cases of unexplained recurrent miscarriage (RM), defined as a series of at least three miscarriages considered unexplained after routine screening (normal uterine cavity, parental karyotypes and endocrine parameters), is associated with immunological disturbances. A series of autoantibodies (Petri et al., 1987; Xu et al., 1990), deficiency of mannan-binding lectin (Kruse et al., 2002), particular class I and class II HLA alleles (Christiansen et al., 1994a; Pfeiffer et al., 2001) and a T-helper type I cytokine bias (Hill et al., 1995) can be found with increased prevalence in these patients. In patients negative for autoantibodies, so-called active immunization with lymphocytes from the partner (partner lymphocyte transfusions (PLT)) or third party donors (donor lymphocyte transfusions (DLT)) have been widely used within and outside controlled trials since 1980. In 1994, a meta-analysis of all placebo-controlled trials (both PLT and DLT trials) showed that allogeneic lymphocyte transfusion (ALT) significantly increased the chance of live birth with 16.3% (95% CI: 4.8–27.8%) among patients with primary RM and no auto- or allo-antibodies, whereas no effect could be detected in patients with secondary RM (RMITG, 1994; Daya et al., 1994). The use of ALT became a quite widespread and accepted treatment until 1999, at which time the results of a large placebo-controlled trial showed that PLT did not increase the chance of live birth compared with placebo but rather tended to decrease it (Ober et al., 1999). This trial has been criticized since the lymphocytes used for transfusions were stored overnight before infusion and because the live birth rate among the immunized patients were much lower than that reported in similar trials. Controversies thus still remain whether ALT is effective or not. Almost all trials on ALT have tested PLT, whereas only three trials have tested DLT. The Cochrane meta-analysis found that the odds ratio (OR) for live birth after PLT was 1.05 (95% CI: 0.75–1.47) and for DLT was 1.39 (95% CI: 0.68–2.82) and concluded that neither treatment provides significant beneficial effect over placebo in preventing further miscarriages (Scott, 2003).

Since the first case-series of treatment of patients with RM with intravenous immunoglobulin (IvIg) were carried out in 1988, seven placebo-controlled trials of this treatment have so far been published. The results have been very divergent, with two trials showing a significant or almost significant treatment effect (Christiansen et al., 1995; Coulam et al., 1995) and five trials showing no overall effect (German RSA/IVIG Group, 1994; Stephenson et al., 1998; Perino et al., 1997; Jablonowska et al., 1999; Christiansen et al., 2002) The Cochrane meta-analysis, which did not look into subgroups of patients, reported that the OR for live birth after IvIg was 0.98 (95% CI: 0.61–1.58) but an updated meta-analysis of the trials (Daya and Gunby, personal communication) showed that the treatment might be efficient in patients with secondary RM since the OR for live birth in IvIg-treated patients was 1.69 (95% CI: 0.72–3.96) which, after inclusion of more studies, may turn out to be significant. On the other hand, there was no effect in patients with primary RM although there was some indication that initiation of treatment before conception may be beneficial in this subgroup.

Based on our own research results, we have after 1992 offered DLT to patients with primary RM and after 2000 IvIg to patients with secondary RM or repeated second trimester intrauterine fetal deaths (IFDs). Since our protocols for immunotherapy diverge very much from those commonly used, especially in terms of the amount of allogeneic cells/IvIg given

and the number of previous pregnancy losses suffered by the patients and our pregnancy outcomes in subgroups of patients are better than those indicated in the meta-analyses, we believe that a survey of our protocols and the results would have general interest and might stimulate the initiation of new trials testing, hopefully, more efficient protocols.

2. Materials and methods

We aim to review the protocols followed and give a survey of the results of our three previously published placebo-controlled trials of DLT and IvIg infusions to women with unexplained RM (Christiansen et al., 1994b, 1995, 2002) undertaken from 1986 to 2000. Furthermore, we review the immunotherapy treatment protocols now followed at the RM Clinic, Rigshospitalet, Copenhagen, Denmark, and report the pregnancy outcomes after immunotherapy in the Clinic since it started in 2000. Our present patient selection criteria for offering the two different forms of immunotherapy are primarily based on the clinical history

Table 1
Algorithm for selection of recurrent miscarriage patients for donor leukocyte therapy (DLT), intravenous immunoglobulin (IvIg) and low-dose heparin (LDH) and low-dose aspirin (LDA) in the Danish RM clinic after 2000

Patient category	DLT	IvIg	LDH/LDA
Primary RM ≥4 First trimester losses No problem to conceive Low titers of autoantibodies	Yes		
Primary RM 3 First trimester losses Problem to conceive Low titers of autoantibodies	Yes		
Primary/secondary RM ≥3 First trimester losses High titers of ACL antibodies			Yes
Primary RM Previous failed treatment with DLT or LDH/LDA		Yes	
Secondary RM ≥4 First trimester losses No problem to conceive		Yes	
Secondary RM 3 First trimester losses Problem to conceive		Yes	
Repeated late IFDs Negative for LAC		Yes	
Repeated late IFDs Positive for LAC		Yes	Yes

ACL: anticardiolipin; LAC: lupus anticoagulant; IFD: intrauterine fetal death.

and the main points are shown in Table 1. If the patients had only suffered first trimester losses and had no problems to conceive, we would, normally not offer immunotherapy since the spontaneous prognosis is considered good and the treatments can have side effects or they are expensive. Data concerning pregnancy outcomes originated from the hospital journals and files created for research purposes.

3. Results

3.1. DLT placebo-controlled trial—protocol

Our placebo-controlled trial of DLT ran from 1987 until 1992 (Christiansen et al., 1994b). Eligible patients were women with at least three previous consecutive miscarriages, normal uterine cavity, normal parental chromosomes and normal endocrinology. Furthermore, the women had to be negative for lymphocytotoxic antibodies, the lupus anticoagulant and they were allowed only to have low titers of autoantibodies. DLTs were undertaken twice before conception with intervals of 1 month. Before each transfusion, 200 ml of blood was drawn from the woman and after 1 h she was, depending on random allocation, either infused intravenously with 150 ml of buffy-coat (leukocyte-enriched blood concentrate) from two red-cell-compatible blood donors or received the same amount of autologous blood. Transfusions were repeated in the same way after 1 month and every fifth month until conception, but no transfusions were given during pregnancy. At each DLT, a total of 1.5×10^9 to 4.6×10^9 white cells were exclusively infused intravenously. This is more than 10 times the number of cells normally given intradermally, subcutaneously and intravenously in PLT protocols.

3.2. DLT placebo-controlled trial—results

In our placebo-controlled trial of DLT (Christiansen et al., 1994b), a significant treatment effect of 38% (95% CI: 7–68%, $P < 0.02$) was found in patients with primary RM whereas no effect at all was found in patients with secondary RM (Fig. 1). There were no significant differences in the proportion of women achieving pregnancy among DLT- and placebo-treated patients, and the time to conception was equal in the two groups. The mean birth weight of children born after DLT among women with primary RM was 3445 g compared with 3000 g among placebo-infused women in the group ($P < 0.05$). The mean birth weight in women with secondary RM was not significantly different between the two groups. Major and minor postnatal complications were found in 8/29 (28%) of the children born after DLT compared with 1/10 (10%) children born after placebo, but looking only at major complications the frequencies were equal in the two groups: 2/29 (7%) versus 1/10 (10%).

3.3. DLT treatment after 2000

After the conclusion of the placebo-controlled trial of DLT in 1992 and, until 2000, we offered this treatment to 25 women with primary RM at Aalborg Hospital and approximately

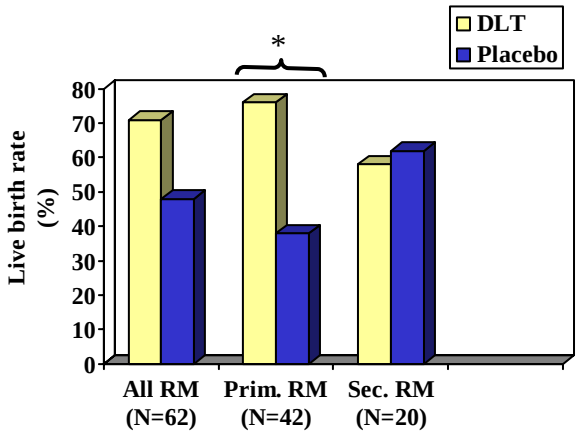

Fig. 1. Live birth rates of patients with recurrent miscarriage according to treatment and clinical history in the Danish placebo-controlled trial (Christiansen et al., 1994b) of donor leukocyte transfusions (DLT) or placebo (autologous blood). * $P < 0.02$.

70% of those who became pregnant had live births. However, since the follow-up rate was poor between 1992 and 2000, in the present study we present only the results after DLT of patients referred to the RM clinic that was established in Copenhagen after 2000 because, in this clinic, it was possible to carry out 100% follow-up of the patients. The results so far from the clinic in Copenhagen are presented in Table 2. The patients are divided into two groups: a group with known subfertility or secondary infertility problems at admission and a group with no previous problems of conception. The frequency of successful pregnancies among those who conceived was similar in the two groups: 50 and 55%, respectively (not statistically significant). The mean observation time after DLT was 1.3 years (range: 3–22 months).

3.4. IvIg placebo-controlled trials—protocol

From 1992 to 1994, we conducted our first placebo-controlled of IvIg in treatment of RM (Christiansen et al., 1995). Eligible patients were women who had had at least three consecutive miscarriages unexplained after routine non-immunogical investigations, but a special demand in this trial was that the patients should have suffered secondary RM or RM

Table 2
Reproductive outcome in patients with primary recurrent miscarriages offered donor leukocyte transfusions after January 2000

	Not pregnant	Miscarried	Birth
Known subfertility problem ($N = 19$)	9	5 (2/3)[a]	5
No subfertility problem ($N = 18$)	7	5 (1/3)[a]	6

[a] Figures in parentheses indicate numbers of fetuses with abnormal karyotypes among all successfully karyotyped abortuses.

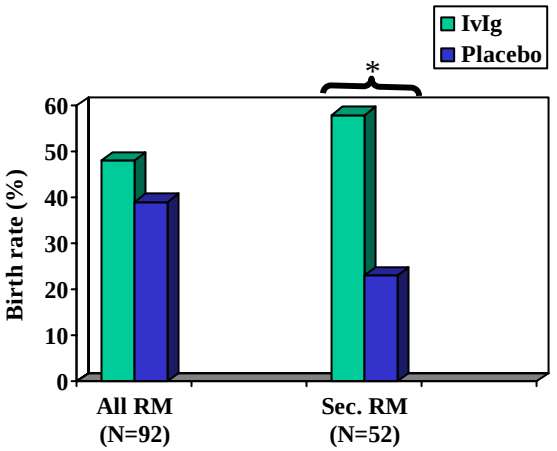

Fig. 2. Live birth rates of patients with recurrent miscarriage according to treatment and clinical history in the combined Danish placebo-controlled trials of intravenous immunoglobulin (IvIg) or placebo (Christiansen et al., 2002). * $P < 0.02$.

including at least one second trimester fetal loss. There were no immunological inclusion or exclusion criteria (except for IgA deficiency). IvIg infusions were started as early as possible in pregnancy (gestational week (GW) 5) and approximately 0.5 g/kg body weight were given weekly until GW 8 and afterwards every second week until GW 34.

In the second placebo-controlled trial of IvIg (Christiansen et al., 2002), the only difference in the inclusion criteria was a demand for at least four previous miscarriages but there was no demand for a previous birth or second trimester loss. Infusions were again started in GW 5 but, until GW 10, weekly infusions of 0.8 g/kg body weight and, after GW 10, every second week were given until GW 26. No concomitant therapies were given.

3.5. IvIg placebo-controlled trials—results

Fig. 2 shows the results of the two combined trials. Among patients with secondary RM, a significant ($P < 0.02$) treatment effect of 34% could be detected whereas in women with primary RM no treatment effect at all could be found. Furthermore, among patients with at least two previous second trimester IFDs included in our pilot project and two placebo-controlled trials, a live birth rate of 7/8 (87%) in IvIg-treated patients compared with 1/9 (11%) in placebo-infused patients was found ($P < 0.01$).

3.6. IvIg treatment after 2000

Table 3 shows the outcome of pregnancies in RM patients treated with IvIg in the RM clinic since 2000. Based on the results from the previous controlled trials, IvIg treatment was offered as the treatment of choice to patients with secondary RM and at least four miscarriages and patients with at least two second trimester IFDs. However, we also offered it to a few patients with only first trimester miscarriages and no previous birth as a last

Table 3
Outcome of pregnancies in women with recurrent miscarriages (RM) treated with intravenous immunoglobulin outside placebo-controlled trials after 1999 according to clinical history and number of previous miscarriages

No. of losses	Live birth rate (live births/all pregnancies)			
	Primary RM (%)	Secondary RM (%)	Repeated late fetal deaths (%)	Total
2–3	0/0 (0)	1/1 (100)	4/4 (100)	5/5 (100)
4	3/5 (60)	1/1 (100)	3/3 (100)	7/9 (78)
>5	4/9 (44)	11/15 (73)[a]	1/2 (50)	16/26 (62)
Total	7/14 (50)*	13/17 (76)	8/9 (89)	28/40 (70)

[a] One karyotypically abnormal abortion in a patient aged 43 years and two biochemical pregnancies in IVF patients among the four miscarriages.

* $P < 0.05$ compared with patients with secondary RM and patients with repeated late fetal deaths.

treatment attempt after other therapies (DLT or heparin/aspirin) had failed (Table 1). In these patients, we normally gave the first IvIg infusion before conception but, during the subsequent pregnancy, infusions were administered after the same protocol as to women with secondary RM. After 2000, our treatment protocol has been modified in order to provide smaller IvIg doses and fewer infusions than in the placebo-controlled trials. The typical infusion schedule has comprised weekly infusions of 25 g IvIg from GW 5 to 9, and afterwards infusions every second week until GW 14 in women with only first trimester miscarriages and to GW 24 in women with second trimester losses. Table 3 shows that the live birth rate was 76% in women with secondary RM, 89% in women with RM including repeated second trimester IFDs, but it was significantly lower and only 50% in women with primary RM ($P < 0.05$). In four of the patients with repeated IFDs, low-dose heparin and aspirin therapy were given in addition to IvIg due to the presence of lupus anticoagulant.

4. Discussion

The results of the placebo-controlled trials carried out in the Danish RM clinics indicated that DLT improves live birth rate in women with primary RM and IvIg improves live birth rate in those with secondary RM and in those with repeated second trimester IFDs (Figs. 1 and 2).

The results obtained from using DLT after conclusion of the randomized trial did not strengthen our conviction as to the effectiveness of this therapy since only 50–55% of the patients who obtained pregnancy had live birth—a significantly lower success rate than that of 76% found in women with primary RM in our controlled trial (Christiansen et al., 1994b). This lower success rate can have two explanations: either the treatment effect was overestimated in the placebo-controlled trial or, more likely, the treatment after 2000 has been offered to a patient group with a poorer spontaneous prognosis than the group included in the controlled trial. Since ALT in theory poses serious side effects (transmission of viruses and prions, suppression of the immune defence against infections (Blajchman, 1997) and maybe a long-term increased risk of some forms of cancer), we have offered it primarily to patients with an estimated poor spontaneous chance for having a child, such as patients with at least four previous miscarriages or RM patients with a concomitant subfertility or

secondary infertility problem (Table 1). The results in Table 2 indicate that the live birth rate seems to be similar in ALT-treated patients with subfertility (5/10, 50%), compared with ALT-treated patients with normal fertility (6/11, 54%) which is encouraging taking into account reports claiming that subfertility is a prognostically negative factor in RM (Cauchi et al., 1995). However, this has to be confirmed in a larger group of patients.

On the other hand, our experience with IvIg after 2000 has fully complied with our results from the placebo-controlled trials: in women with secondary RM, the observed success rate of 76% has been even higher than the success rate in the placebo-controlled trials (58%) although the new patients had suffered an even higher number of previous miscarriages than those in the randomized trials (Christiansen et al., 2002). The success rate in patients with repeated IFDs was still very high (89%) and the success rate in patients with primary RM was still significantly lower than in the other groups. The new results strongly support the effectiveness of IVIG in women with secondary RM or repeated late IFDs.

The discussion about the efficiency and the dangers of offering immunotherapy to women with unexplained RM has now been ongoing for several years. The opponents of this therapy refer to the Cochrane meta-analysis of relevant placebo-controlled trials showing that none of the tested immunotherapy modalities seems to improve live birth rates significantly compared with placebo (Scott, 2003) and the fact that the initial theories trying to explain its possible effects have not been substantiated (Coulam, 1992).

Those who believe it is too early to abandon these therapies put forward three main arguments.

Firstly, some of the treatment modalities have only been tested in few trials, e.g. DLT has only been tested in three trials including 156 patients, and the inclusion of more patients in the meta-analysis may result in the odds ratio for live birth after DLT, which was 1.39 (95% CI: 0.68–2.82), becoming statistically significant.

Secondly, the trials of immunotherapy are all too heterogenous in their inclusion of patients and treatment protocols to allow any firm conclusions to be taken in the meta-analyses. Concerning active immunotherapy, in Section 3 we provided information that DLT, as offered in our clinic, is a completely different way of modulating the immune system, in terms of the amount, the origin and the route of administration of the immunizing agent, than PLT. There is evidence that intravenous administration of high doses of an antigen, in the absence of additional costimulatory signal (as done by DLT in our clinic), is a better way to induce tolerance than subcutaneous/intradermal administration of smaller doses of antigen normally used in PLT (Wood et al., 2001). Concerning passive immunization with IvIg, the significantly larger doses of IvIg given more frequently in our trials compared with other trials (Fig. 3) may also partly explain the good pregnancy outcomes in our patients with secondary RM. Furthermore, the patient populations were very different in the different trials; in our two placebo-controlled trials, 74–100% of the patients had more than four previous miscarriages compared with only from 19% (German RSA/IVIG Group, 1994) to 53% (Stephenson et al., 1998), of the patients in other placebo-controlled trials. In two trials (German RSA/IVIG Group, 1994; Perino et al., 1997), patients with secondary RM were excluded whereas in the other trials patients with secondary RM comprised more than half of the patients. This heterogeneity may have great importance for the possibility to find an effect of IvIg immunotherapy since there are many indications that women with at least four to five miscarriages and women with secondary RM more often have an immunological

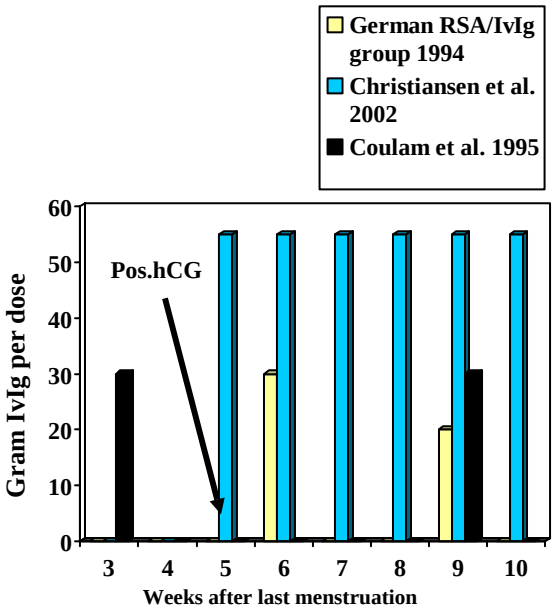

Fig. 3. Details of the infusion protocols from three placebo-controlled trials of intravenous immunoglobulin (IvIg) in patients with recurrent miscarriage.

background for their problem than patients with fewer miscarriages and no previous birth (Christiansen, 1996; Kruse et al., 2002).

To conclude that immunotherapy is inefficient in the treatment of RM based on data from the available trials and meta-analysis would be comparable to doing a study of treating women with 3-year infertility, regardless of the cause, with ovarian stimulation using a fixed low FSH dose. Such a study would not be able to show any significant effect of FSH therapy over placebo. However, if the patients in such a hypothetical trial were subdivided according to the causes of infertility, and only those with anovulation and no other infertility causes were treated with adequate FSH doses adjusted by monitoring of the follicle growth, a significant treatment effect would be found. Similarly, when RM patients are divided into subgroups according to their clinical and paraclinical features and offered adequate treatment, the chance of finding a significant treatment effect is improved. In our trial (Christiansen et al., 1994b) and the RTMIG meta-analysis (RMITG, 1994), allogeneic lymphocyte immunization only proved efficient in women with primary RM and in our trials of IvIg, only women with secondary RM benefited from the treatment.

The third argument in favor of continuing research in the efficacy of immunotherapy to women with RM comes from new insight in the background for development of immmunological tolerance. Table 4 lists a number of theories trying to explain why active immunotherapy with blood transfusions and passive immunotherapy with IvIg induce tolerance, improve the clinical outcome after organ transplantation or in autoimmune disorders, and may work in the prevention of pregnancy loss (Kwak et al., 1996). Several of these theories have so far not been substantiated, e.g. in our placebo-controlled trials of IvIg

Table 4
Theories trying to explain the effect of active and passive immunotherapy in the induction of tolerance in transplantation, autoimmune disease or recurrent miscarriage

	Theory
Active immunotherapy (lymphocyte transfusions)	Production of anti-paternal antibodies or blocking antibodies Dampening of NK cell activity Modification of cytokine production Establishment of microchimerism
Passive immunotherapy (intravenous immunoglobulin)	Suppression of autoantibodies Neutralization of autoantibodies Dampening of NK cell activity Modification of cytokine production Inhibition of complement binding and activation Fc receptor modulation and blockade Inhibition of superantigens Modulation of adhesion molecules on T lymphocytes Induction of apoptosis of activated cytotoxic lymphocytes

(Christiansen et al., 1995, 2002) we were not able to detect any decrease in the levels of a series of autoantibodies from GW 5 to 12—on the contrary, the concentration of IgG anticardiolipin antibodies seems to increase in IvIg-treated patients which probably is due to anticardiolipin antibodies in the IvIg preparations. Several of these mechanisms, e.g. induction of apoptosis of activated lymphocytes (Prasad et al., 1998) or establishment of donor cell microchimerism (Starzl et al., 1993), have so far not been investigated in RM patients receiving active or passive immunotherapy. Relevant research should be done to clarify whether induction of changes in these parameters plays a role in the possible prevention of miscarriage by immunotherapy.

In conclusion, the published research in the field of immunotherapy in the prevention of RM neither allows rejection or acceptance of this treatment. More large placebo-controlled studies of, in particular, DLT exclusively given intravenously should be done among patients with primary RM without high titers of auto- or allo-antibodies. Furthermore, more placebo-controlled studies of IvIg should be done among patients with secondary RM. Patients undergoing immunotherapy or placebo-treatment should be monitored for changes of some of the immunological parameters that have drawn attention during recent years (Table 4). Finding a significant association between immunotherapy-induced changes in these parameters and successful pregnancy outcome will stress the view that immunotherapy given in the right way to the right patients indeed has therapeutic effect.

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