

Effect of tumor necrosis factor- α blocker (infiximab) on blastocyst development in vitro

Infiximab does not have any toxic effects on early cleaving embryos. (*Fertil Steril*® 2004;81:1704–6. ©2004 by American Society for Reproductive Medicine.)

Tumor necrosis factor- α (TNF- α) has gained recent attention in the pathophysiology of autoimmune disease. It is a pleiotropic cytokine; these exert cytotoxic as well as differentiation and growth modulator activities on many different target cells (1). It has a range of beneficial and injurious effects, depending on the quantity produced, their tissue localization, and the local activity of TNF-binding proteins and their hormonal and cytokine milieu (2). Tumor necrosis factor- α is produced by neutrophils, activated lymphocytes, and natural killer cells, and it is a major product of activated macrophage.

Tumor necrosis factor- α activates inflammatory leukocytes, resulting in production of other proinflammatory cytokine such as interleukin (IL)-1, IL-6, and IL-8 and additional TNF- α (3). It stimulates the proliferation of endometriotic stromal cells, endometrial cell adhesion, as well as matrix metalloproteinase expression, both of which are necessary events in the initial development of the disease (3). Numerous studies have demonstrated that TNF- α levels are elevated in the peritoneal fluid of patients with endometriosis (1, 4) and that these higher concentrations correlate with the stage of the disease.

Endometriosis shares many similarities with autoimmune diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis. Like classical autoimmune disease, endometriosis has been associated with polyclonal B-cell activation, immunological abnormalities in T-cell and B-cell functions, increased apoptosis, tissue damage, multiorgan involvement, familial occurrence, possible genetic basis, involvement of environmental cofactors, female preponderance, and association with other autoimmune disease (5). Because it is well established that endometriosis is a disease that is associated with immune or inflammatory disorders, immunomodulator drugs, specifically anti-TNF- α therapies, may be viable options for use in the treatment of endometriosis. Consequently, targeting TNF- α may be a logical starting point in the development of novel treatments for endometriosis, as proven by experimental studies (1, 6).

Infiximab (Remicade; Centocor, Inc., Malvern, PA) is currently approved for the treatment of rheumatoid arthritis and Crohn's disease. It is a chimeric monoclonal antibody that binds both soluble and membrane forms of TNF- α and neutralizes its biological effects (7). With accumulating evidence, clinical use of infiximab for endometriosis patients is a strong possibility in the near future. Little knowledge is available regarding possible effects of infiximab on early embryonic development. We sought to assess whether infiximab was embryotoxic by using a mouse embryo model.

We used infiximab that was reconstituted with 10 mL of sterile water prepared with an aseptic technique (concentration, 100 mg/10 mL). During controlled clinical trials, maximum infiximab plasma levels do not exceed 10 μ g/mL, even at maximum doses (7). Infiximab was dissolved in human tubal fluid (HTF) media (Irvine Scientific, Santa Ana, CA) to give working concentrations of 1, 10, 50, 100, 200, and 400 μ g/mL. The culture dishes containing 1 mL of media at each concentration were incubated for 6 hours (equilibration) at 37°C and 5% CO₂.

Frozen straws containing 2-cell mouse embryos (Embryotech Laboratories, Inc., Wilmington, MA) were exposed to room temperature for 2 minutes. Each straw was bisected between the lower heat seal and the column of medium. By using the stylet, the contents of the straw were flushed as a single drop into a sterile culture dish (Allegiance Health Care, Inc., McGaw Park, IL). Thawed mouse embryos were pooled and randomly distributed between the following seven groups: group A was composed of HTF supplemented with infiximab (1 μ g/mL), group B was HTF supplemented with infiximab (10 μ g/mL), group C was HTF supplemented with infiximab (50 μ g/mL), group D was HTF supplemented with infiximab (100 μ g/mL), group E was HTF supplemented with infiximab (200 μ g/mL), group F was HTF supplemented with infiximab (400 μ g/mL), and a control group (group G) composed of plain HTF media. The numbers of embryos were from 30 to 50 per group. Blastocyst development rates (BDRs) were checked after 72 hours of incubation. The BDR was calculated by dividing the number of blastocysts by the total number of embryos that were incubated.

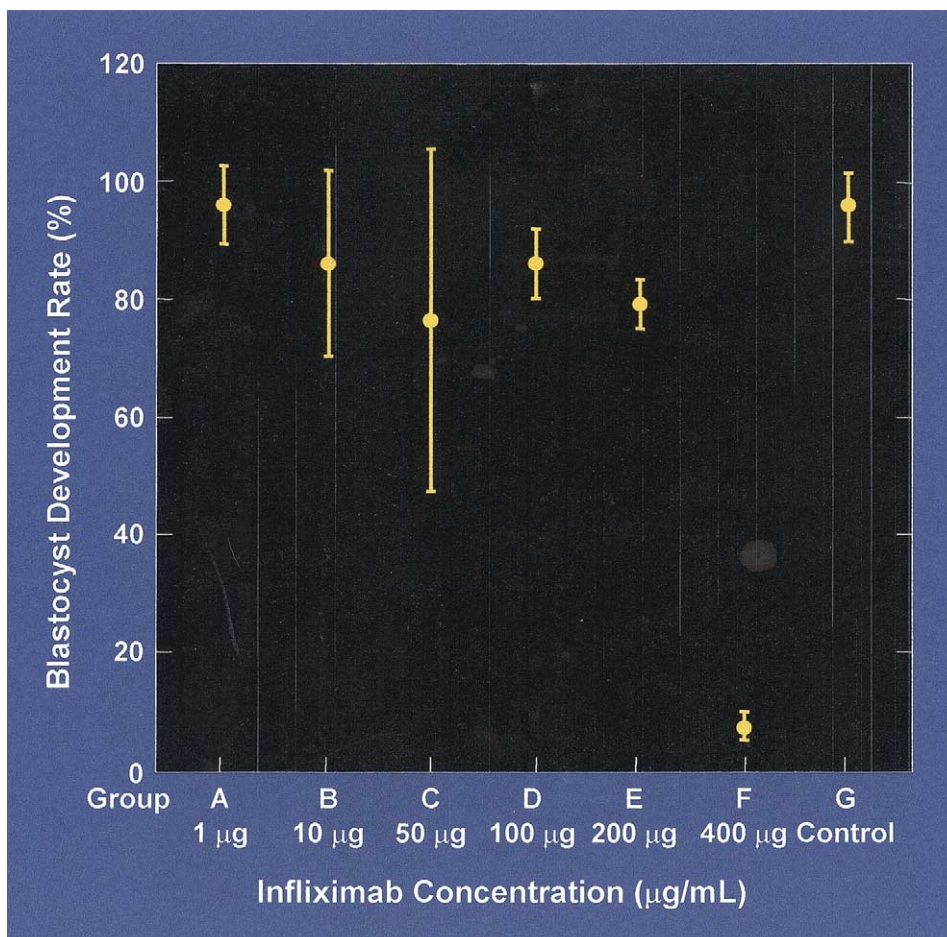
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FIGURE 1

Effect of different concentrations of infliximab on the blastocyst development rate of mouse embryos. The bar represents the median and the range of data.



Noriega. Effect of TNF- α blocker. *Fertil Steril* 2004.

The relationship between BDR and concentration was assessed with repeated-measures logistic regression by using generalized estimated equation methodology with a compound symmetry correlation structure. The sample size was sufficient to detect whether a specific concentration reduced the odds of development by a factor of 2.5. Statistical significance was assessed by using two-tailed $P < .05$. Statistical computations were performed with SAS, version 8.1 (SAS Institute Inc, Cary, NC).

Blastocyst development rates were 96.3%, 86.7%, 77.3%, 86.7%, 80%, 10%, and 96% for the seven groups, respectively (Fig. 1). Blastocyst development rates of all groups but group F were comparable with the control group (group G). On the other hand, group F had significantly lower BDR compared with the control group ($P < .0001$). The embryos in group F were arrested at early stages of development and contained extensive fragmentation.

Although substantial evidence indicates that endometriosis may share many similarities with autoimmune diseases, so far it is primarily treated with compounds that create a hypoestrogenic

environment that is associated with adverse and unpleasant effects. Thus, novel therapeutic approaches including anticytokines may provide a treatment modality that targets the exact etiological factors rather than the symptoms. The results of using TNF blockers in an endometriosis animal model were very encouraging and provide evidence for potential clinical use.

D'Antonio et al. (6) assessed whether recombinant human TBP-1, a soluble form of TNF receptor type I, reduced the size of endometriotic-like foci in a rat model. Endometriotic foci were reduced in size by 33% and 64%, respectively, 2 and 9 days after the last dose. In another study, Iwabe et al. (1) showed that the stimulatory effect of TNF- α in the proliferation of endometrial stroma cells was abolished by adding anti-TNF- α antibody in vitro. Similarly, peritoneal fluid from an endometriosis patient enhanced the proliferation of autologous and heterologous endometrial cells cultures, and the soluble TNF receptor (Etanercept, Immunex, Seattle, WA) blocked this effect (8). In a baboon animal model, D'Hooghe et al. (9) reported neutralization of TNF- α with recombinant human TBP-1,

which inhibited the development of endometriotic lesions and prevented adhesion formation in animals that were treated with that anticytokine.

There are no data available about possible effects of infliximab on early embryonic development. This study provides the first evidence that infliximab does not affect BDR at concentrations similar to the maximum in vivo levels. Embryotoxic effects of infliximab appear only at a concentration of 40-fold, its maximum in vivo serum level. However, biodilution or bioconcentration of infliximab in the female reproductive tract could happen. This may result in concentrations that are far higher than serum levels. Infliximab does not appear to have any toxic effects on early cleaving embryos. Given the future potential of TNF- α blocker in endometriosis therapy, this preliminary report provides provisional reassuring data supporting the implementation of TNF- α blocker in clinical practice.

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References

1. Iwabe T, Harada T, Tsudo T, Nagano Y, Yoshida S, Tanikawa M, et al. Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J Clin Endocrinol Metab* 2000;85:824-9.
2. Vignali M, Infantino M, Matrone R, Chiodo I, Somigliana E, Busacca M, et al. Endometriosis: novel etiopathogenetic concepts and clinical perspectives. *Fertil Steril* 2002;78:665-78.
3. Nothnick WB. Treating endometriosis as an autoimmune disease. *Fertil Steril* 2001;76:223-31.
4. Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, et al. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. *Hum Reprod* 2002;17:426-31.
5. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. *Fertil Steril* 2001;75:1-10.
6. D'Antonio M, Martelli F, Peano S, Papoian R, Borrelli F. Ability of recombinant human TNF binding protein-1 (r-hTBP-1) to inhibit the development of experimentally-induced endometriosis in rats. *J Reprod Immunol* 2000;48:81-98.
7. Markham A, Lamb HM. Infliximab: a review of its use in the management of rheumatoid arthritis. *Drugs* 2000;59:1341-59.
8. Braun DP, Ding J, Dmowski WP. Peritoneal fluid-mediated enhancement of eutopic and ectopic endometrial cell proliferation is dependent on tumor necrosis factor-alpha in women with endometriosis. *Fertil Steril* 2002;78:727-32.
9. D'Hooghe TM, Cuneo S, Nugent N. Recombinant human TNF binding protein-1 (r-hTBP-1) inhibits the development of endometriosis in baboons: a prospective, randomized, placebo-and-drugs-controlled study. *Fertil Steril* 2001;76(Suppl):S1.