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The Ability of Anti-TNF-α Antibodies Produced in Sheep Colostrums

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ABSTRACT

The present study was performed to elucidate the ability of anti-TNF-α antibodies produced in sheep colostrums to neutralise TNF-α action in a cell-based bioassay and in a small animal model of intestinal inflammation.

Colostrums from sheep immunized against TNF-α significantly inhibited TNF-α bioactivity in the cell based assay. The higher than anticipated variability in the two animal models precluded assessment of the ability of antibody to prevent TNF-α induced intestinal damage in the intact animal.

(**Key words** : anti-TNF-α antibody, inflammation)

INTRODUCTION

Many immunological abnormalities have been described in Inflammatory bowel diseases(IBD) and is comprised of two major disorders demonstrate a rising incidence in many countries; Crohn's disease and ulcerative colitis. These diseases have a worldwide incidence of between 1 and 10 cases per 100,000 per year[1]. These disorders have distinct pathologic and clinical characteristics but their pathogenesis remains poorly understood. Intestinal inflammation is also observed with food allergies, gastrointestinal infections, celiac disease and other autoimmune disorders. For instance, up to 50% of patients suffering from arthritic conditions also suffer from intestinal inflammation[2]. For this reason, modulation of IBD also could affect other related diseases. Even though, no clear shot primary defects have yet been described, some therapeutic trials have targeted either immunocompetent cells or overproduction of cytokines. The immunology of mucosal inflammation in both disease is characterized by an overwhelming preponderance of pro-inflammatory cytokine expression with an apparent inability to adequately downregulate immune activation.

The pro-inflammatory cytokine, TNF-α is produced primarily by activated macrophages within the inflamed tissue involved in systemic inflammation and is a member of a group of cytokines that all stimulate the acute phase reaction. TNF-α causes apoptotic cell death, cellular proliferation, differentiation, inflammation, tumorigenesis, and viral replication. TNF-α's primary role is in the regulation of immune cells, overproduction of TNF-α have been implicated in a variety of human disease, as well as IBD and cancer[3]. Anti-tumor necrosis factor(Anti-TNF) agents are efficacious in treating IBD, but not all are equally effective. Treatment with infliximab, a mouse- derived monoclonal antibody to TNF-α, by intravenous infusion has been shown in several studies to reduce the symptoms of active Crohn's disease in patients which had not responded to conventional therapies[4-6]. However, these data need to be confirmed and the potential side effects of these treatments must be further considered. These drugs need to be more precisely defined in particular compared to corticotherapy. Inflammatory bowel diseases are chronic inflammatory and frequently relapsing diseases of the gut that ultimately lead to destruction of the intestinal tissue. Recent evidence suggests that a pathologic activation of the mucosal immune system in response to antigens is a key factor in the pathogenesis of IBD. Furthermore, changes in cell migration and cytokine production appear to contribute to the perpetuation of IBD and the postoperative recurrence of Crohn's disease. It is possible that oral administration of anti-TNF-α antibodies that act directly in the gut, could provide the same benefits without the associated problems.

In this study, the hypothesis that anti-TNF-α antibodies produced in sheep colostrums modulate TNF-α action in a cell-based bioassay and in a small animal model of intestinal inflammation. This result will contribute to further study in this

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field as a basic data for oral administration of anti-TNF-α antibodies management of intestinal inflammation.

MATERIALS AND METHODS

1. *In vitro* Study : Antibody Activity in Cell Bioassay

The inhibition of TNF-α activity in the mouse fibrosarcoma WEHI-13 VAR cells(ATCC CRL-2148) bioassay by antibody from two sheep using the method described by Espevik and Nissen-Meyer[7] with some minor modifications. The WEHI-13 VAR cell line was found to maintain its high sensitivity and provided a stable bioassay system to detect and measure mouse and recombinant tumor necrosis factors(TNF-α and lymphotoxin. RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 90%; fetal bovine serum, 10%).

To verify the inhibition of TNF-α(0.5 ng) activity in the WEHI-13 VAR bioassay by antibody from two sheep, samples take at 24 h were incubated with 1 in 200 to 1 in 600,000 dilution of sheep TNF-α polyclonal antiserum, except for control sample that was diluted 1 in 5,000 in the assay.

2. *In vivo* Study : Rat Trial 1, 2

1) Rat Trial 1

A pilot trial to establish dosage, method of administration and timing was initially conducted to test effectiveness and repeatability of treatment to induce inflammation.

Experimental animal were performed for 4 groups as follow :

A. Uninduced control, no Ig
B. Induced inflammation, no Ig
C. Induced inflammation + normal sheep Ig preparation 600 mg/kg/day
D. Induced inflammation + Anti-TNF-α sheep Ig preparation 600 mg/kg/day

All rats were fed on a standard maintenance diet throughout the experiment with rats in groups C & D receiving 600 mg Ig/kg/day twice daily by oral gavage for 2 days prior to inducing intestinal inflammation. Total volume at each gavage was 1 ml. On the third day, experimental intestinal inflammation was induced by injection of platelet-activating factor(PAF) and LPS into the tail vein. The rats were restrained in plastic vessels, warmed for 15 minutes on a 37℃ warming plate to increase circulation in the tail and the PAF/LPS mixture injected directly into the tail vein. Up to 100 uL saline solution containing 2.5 mg/mL BSA, 10 ug/mL PAF(PAF stock at 2 mg/mL in CH_3Cl stored −70℃ dried and dissolved in saline/BSA) and 2 mg/mL lipopolysaccharide(LPS, *Salmonella typhosa*) was injected(final volume determined by weight of animal) using 1.0 mL tuberculin syringe with 26 gauge needle. The total volume injected was calculated to deliver 4 ug/kg PAF and 0.8 mg/kg LPS as according to Gonzalez-Crussi and Hsueh[8] this an optimal dose.

Two hours following induction, rats were enthanased using CO_2 and their intestines removed for examination. Intestinal damage was assessed histologically according to Caplan et al[9]. Briefly, a score of 1+ will be assigned to sections normal in appearance with intact villi, 2+ for partial necrosis of the villi, 3+ for necrosis of the entire villi, 4+ for evidence of transmural damage.

2) Rat Trial 2

An initial pilot trial was conducted on four male Sprague-Dawley rats(approx 150 g). Colitis was induced by administering 30mg trinitrobenzenesulphonic acid(TNBS) in 0.25 mL of 50% ethanol as an anema. This induces acute diarrhea and weight loss within 24~48 hours. Weight and general appearance of the rats were monitored every 4~8 hours over the next 48 hours. Rats were euthanased with CO_2 and their colon removed. Intestinal damage was assessed histologically according Caplan et al[9].

RESULTS

1. *In vitro* Study : Antibody Activity in Cell Bioassay

Antibody at 1 in 10,000 dilution was able to completely inhibit TNF-α activity in the cell bioassay. The antibody from the same sheep, but different milkings, exhibited some variability in inhibition of TNF-α activity, but were all greater than the control sample. This latter sample was collected from a sheep immunized similar to the TNF-α, but with *Candida albicans* antigen.

2. *In vitro* Study : Susceptibility to Proteolytic Digestion

The proteolytic susceptibility study determined that use of 0.1M sodium bicarbonate buffer, pH 9.4 provided greatest protection against protein breakdown by pepsin out to al least 60 minutes(data not shown).

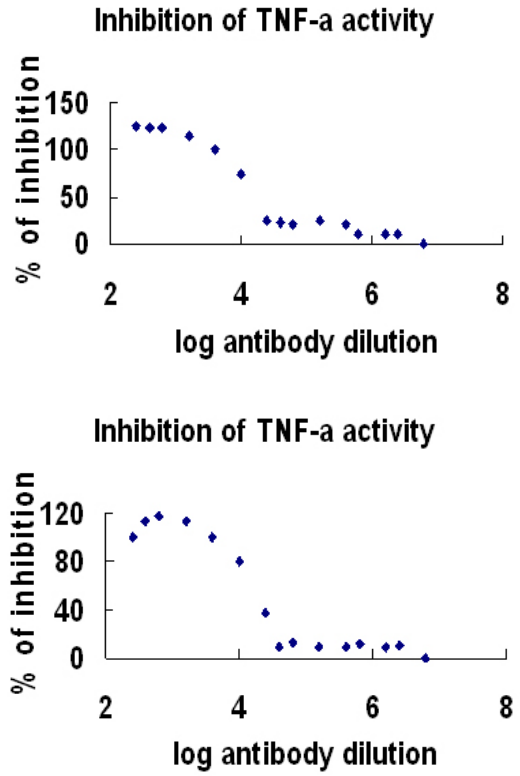

Fig. 1. The inhibition of 0.5 ng TNF-α activity in the WEHI-13 VAR bioassay by antibody from two sheep. Antibody containing samples were diluted 1 in 200 to 1 in 600,000 in the assay.

3. *In vivo* Study : Rat Trial 1

The rats in the pilot trial became lethargic within 15~30 minutes of the injection of PAF and LPS, remaining in this de-pressed state until euthanased 2 hours after injections. Examination of intestine revealed presence of some blood in the lumen with some isolated regions of obvious damage. The main trial was then conducted, in which two groups of rats received anti-TNF-α Ig or Ig from normal sheep and one group no Ig before administration of PAF and LPS. In contrast to the pilot trial, 3 of 4 animals in each group, including those that were not given Ig, died within 60 minutes following injection PAF and LPS. The one rat from each group that did not die before 2 hours, showed significant amount of blood in the intestinal lumen. The intensity of the inflammatory response was far greater than in the pilot trials and we were unable to differentiate any effect of antibody to reduce the impact of PAF and LPS. It was concluded that the degree of inflammation was beyond the ability of any treatment to prevent.

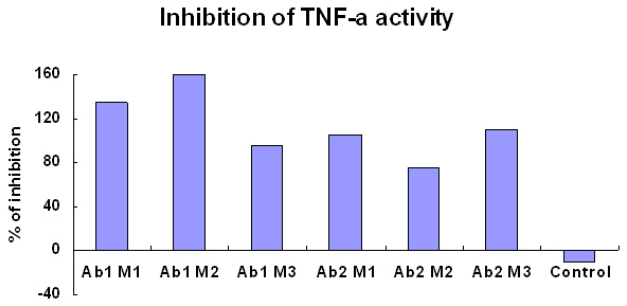

Fig. 2. The inhibition of 0.5 ng TNF-α activity in the WEHI-13 VAR bioassay by antibody from two sheep from different milkings. All samples were diluted 1 in 10,000 in the assay, except for control sample that was diluted 1 in 5,000.

4. *In vivo* Study : Rat Trial 2

Another animal model, involving instillation of TNBS via the rectum to induce colitis was investigate. Several papers had described instillation of 30 mg TNBS in 0.25 mL of 50% ethanol via the rectum resulted in acute diarrhea and weight loss within 24~48 hours. An initial trial with 4 rats was conducted to assess the utility of the model. Only two of the rats exhibited an average weight loss of 18 g(12% body weight) and mild pathology in the colon. In contrast, the other two gained an average 7 g(5% of body weight) over the two days. This weight gain was slightly lower than expected of normally growing rats of this stage of development, but still within the normal range. Based on this pilot trial, the TNBS model would seem to present the same problems as the PAF and LPS model, with a larger variability in response than anticipated.

DISCUSSION

Inflammatory process leads to the well-known mucosal damage and therefore a further disturbance of the epithelial barrier function, resulting abnormal intestinal wall function, even further accelerating the inflammatory process[10]. Despite of the records, etiology and pathogenesis of IBD remain rather unclear. There are many studies over the past couple of years have led to great advanced in understanding the IBD and their underlying pathophysiologic mechanisms. From the current understanding, it is likely that chronic inflammation in IBD is due to aggressive cellular immune responses including increased serum concentrations of different cytokines. Therefore, targeted molecules can

be specifically eliminated in their expression directly on the transcriptional level. Interesting therapeutic trials are expected against adhesion molecules and pro-inflammatory cytokines such as TNF-α. The future development of immune therapies in IBD therefore holds great promises for better treatment modalities of IBD but will also open important new insights into a further understanding of inflammation pathophysiology.

Treatment of cytokine inhibitors such as Immunex(Enbrel) and J&J/Centocor(Remicade) which are mouse-derived monoclonal antibodies have been shown in several studies to modulate the symptoms of patients, however, theses TNF inhibitors also have an adverse effect immune-related problems and also are costly and must be administered by injection. Because of the eventual development of unwanted side effects, these two products are used in only a select patient population.

The present study was performed to elucidate the ability of TNF-α antibodies produced in sheep colostrums to neutralise TNF-α action in a cell-based bioassay and in a small animal model of intestinal inflammation.

In vitro study, inhibitory effect of anti-TNF-α antibody from the sheep was determined by cell bioassay. The antibody from the sheep at 1 in 10,000 dilution was able to completely inhibit TNF-α activity in the cell bioassay. The antibodies from the same sheep, but different milkings, exhibited some variability in inhibition of TNF-α activity, but were all greater than the control sample. *In vivo* study, the degree of inflammation was severe to experiment, despite of the initial pilot trial, main trial 1 was unable to figure out of any effect of antibody to reduce the impact of PAF and LPS. Main rat trial 2 resulted no significant symptoms like characteristic acute diarrhea and weight loss of colitis.

This study suggested that colostrums from sheep immunized against TNF-α significantly inhibited TNF-α bioactivity in the cell based assay. And the higher than anticipated variability in the two animal models precluded assessment of the ability of antibody to prevent TNF-α induced intestinal damage in the intact animal. Further study will require to find out an alternative animal model, which is more acceptable to test anti-TNF-α IgA therapy for reducing the impact of inflammation on gut dysfunction. And subsequent pre-clinical and clinical testing also need generation of more antibody as current supplies are low.

요 약

본 연구는 양(羊)의 초유(初乳)에 발현시킨 TNF-α 항체를 이용하여 염증반응에서 생성된 TNF-α의 활성억제를 통한 염증반응 완화능을 세포단계의 생물검정과 장 염증이 유도된 동물모델을 통해 검증하였다.

실험 결과, 면역반응을 통해 생성된 양(羊)의 초유(初乳)에 함유된 TNF-α 항체는 WEHI-13 VAR 세포의 TNF-α 활성을 유의적으로 억제함을 확인할 수 있었다. 동물실험 1의 경우, 예상되는 TNF-α 항체의 염증반응 억제 효과보다 유도된 염증반응의 정도가 강하였고, 동물실험 2의 경우 대장염 유도에 대해 실험동물간의 민감성 차이를 나타내었다.

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