An Orally Active Alpha 4 Integrin Antagonist AJM300 Prevents the Development of Experimental Colitis Induced by Adoptive Transfer of IL-10 Deficient CD4⁺ T cells in Mice.

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Summary

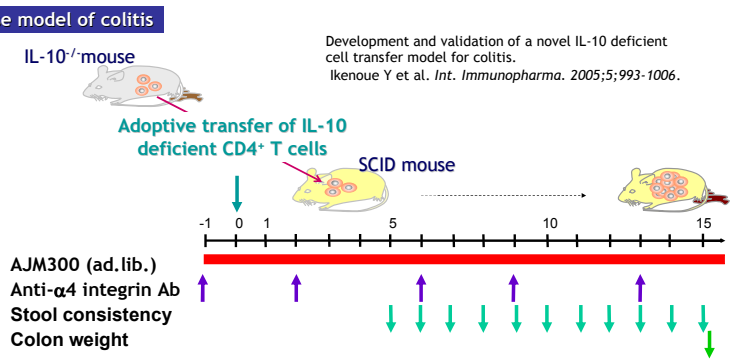
- AJM300, an orally active small molecule alpha 4 integrin antagonist, is currently being developed for IBD in Japan.
- AJM300 was effective in a mouse model of colitis induced by adoptive transfer of IL-10 deficient CD4⁺ T cells.
- The efficacy of AJM300 at 1% of diet was shown to be comparable to the maximum efficacy of alpha 4 integrin blockade.
- AJM300 might become the promising oral drug for the treatment of patients with IBD.

Introduction & Purpose

- Inhibition of leukocyte trafficking with anti-alpha 4 integrin antibody has been clinically validated as a therapeutic approach for inflammatory bowel disease (IBD). However, the orally effective 'anti-alpha 4 integrin therapy' may be more attractive in clinical practice.
- AJM300, an orally active small molecule alpha 4 integrin antagonist, is currently being developed for IBD in Japan. The results of the clinical study were presented at GASTRO2009 on 23 November (OP054).
- The aim of this study was to evaluate the efficacy of AJM300 in a mouse model of colitis induced by adoptive transfer of IL-10 deficient CD4⁺ T cells. In this model, chronic intestinal inflammation invariably developed and diarrhea was exhibited within 2-3 weeks.

Methods

Mouse model of colitis



Development and validation of a novel IL-10 deficient cell transfer model for colitis.
Ikenoue Y et al. *Int. Immunopharma. 2005;5;993-1006.*

- CD4⁺ T cells were isolated from spleens and mesenteric lymph nodes of diseased IL-10$^{-/-}$ Balb/c mice, and adoptively transferred to female C.B-17/Icr-scid/scid Jcl (SCID) mice on day 0. AJM300 was orally given at the concentrations of 0.03%, 0.1%, 0.3% and 1% in the diet, and anti-mouse alpha 4 integrin monoclonal antibody (PS/2) was intraperitoneally administrated at the dose of 10 mg/kg twice a week, and the first-dosing was started on day -1. Each group consisted of 8 animals.
- The stool consistency (0:normal beaded stool, 1:soft stool, 2:diarrhea) was recorded everyday, starting from day 5. On day 15, the colons were removed and weighed.
- Both the infiltration of T cells (CD3⁺ cells) in the colonic lamina propria and the expression of MAdCAM-1 were analyzed by the immunofluorescence staining. The concentrations of pro-inflammatory cytokines (IFN-gamma, IL-17, TNF-alpha and MCP-1) in the homogenized colon samples were measured.

Results

Stool consistency

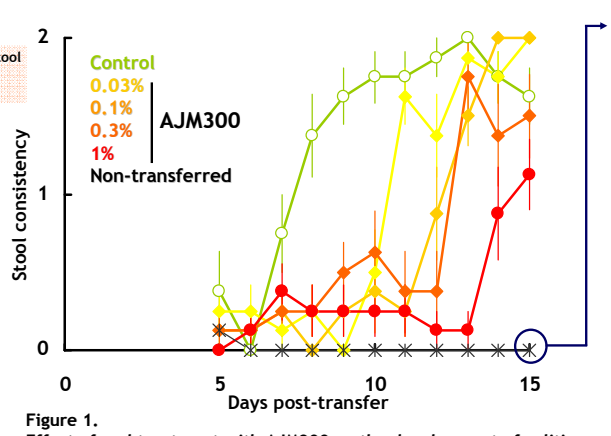
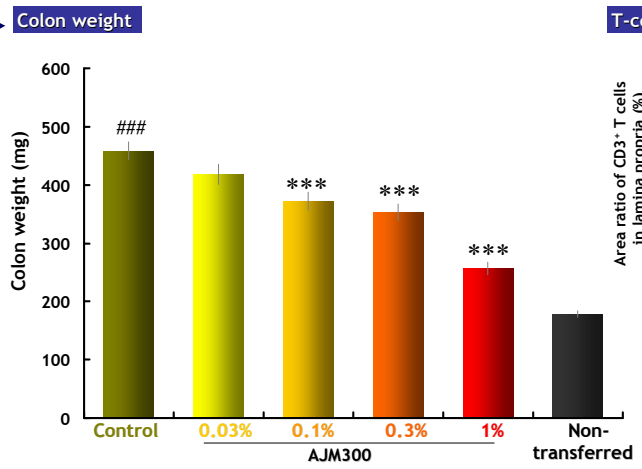


Figure 1.
Effect of oral treatment with AJM300 on the development of colitis.
Data represents mean (n=8).

Colon weight



T-cell infiltration into the lamina propria

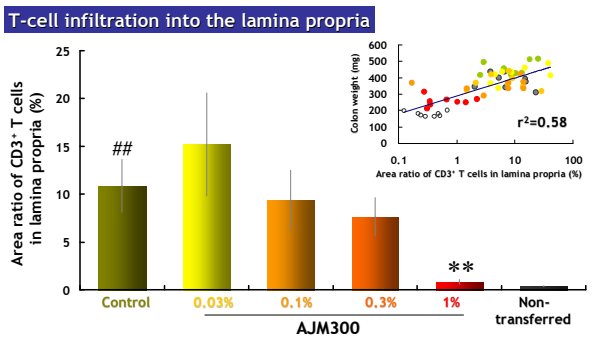


Figure 2.
Effect of oral treatment with AJM300 on the colon weight (left) and CD3⁺ T cell infiltration into the lamina propria (right).

Data represents mean±SE (n=8).
Colon weight; ###p<0.001 vs Non-transferred by Welch's t test.
***p<0.001 vs Control by Dunnett's test
T cell; ##p<0.01 vs Non-transferred by Welch's test. **p<0.01 vs Control by Steel test

Histopathological examination and Immunohistochemical staining

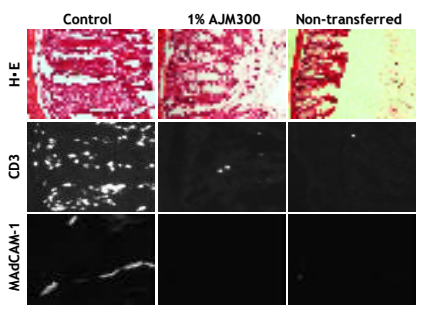


Figure 3.
Histopathological examinations of the day 15 colons from mice treated with control (left), AJM300 at 1% in the diet (middle) and non-transferred (right). Hematoxylin and eosin staining (upper), and immuno-histochemical stainings for CD3-positive cells (middle) and MAdCAM-1 (lower).

Pro-inflammatory cytokines in the colon tissue

Table 1. Concentrations of pro-inflammatory cytokines in the homogenized colon samples on day 15.

	IFN-gamma	IL-17	TNF-alpha	MCP-1
Control	28±4	35±2	25±2	150±10
1% AJM300	13±3 *	9.5±4.4###	13±3 *	100±20
Non-transferred	1.7±0.5	N.D.	1.5±0.6	19±11

(pg/mg)

Data represents mean±SE (n=7 or 8).
*p<0.05 vs control by student's t test
###p<0.001 vs control by Welch's test
N.D.; Not detected

Colonic inflammation and the increased expression of MAdCAM-1 were observed in control mice on day 15. Prophylactic oral treatment with AJM300 significantly prevented the development of colitis in mice at 1% in the diet, accompanied with 96% decrease in CD3⁺ T cell infiltration into the lamina propria. The colon weight was positively correlated with T-cell infiltration. AJM300 at 1% in the diet prevented the increase in the concentrations of pro-inflammatory cytokines in the colon.

Stool consistency

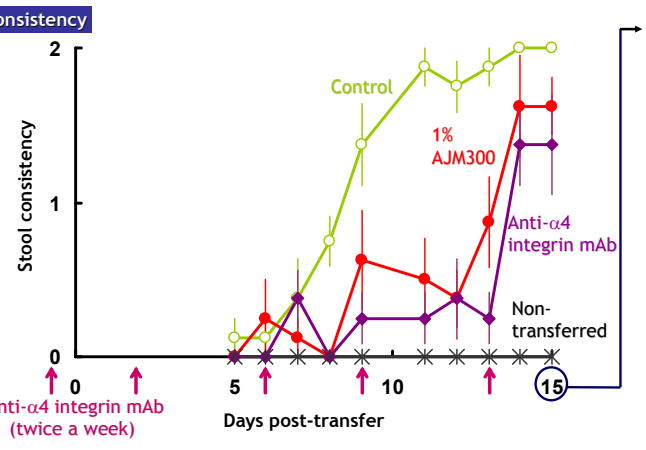
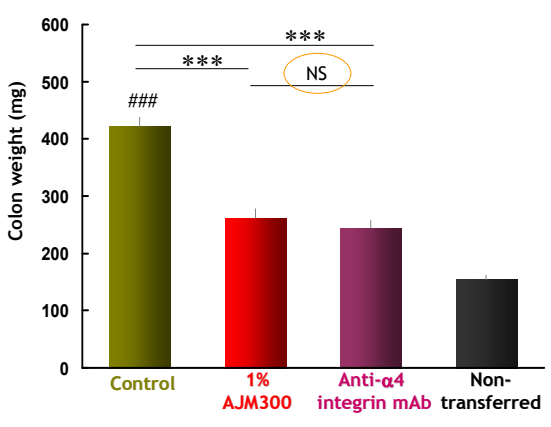


Figure 4. Effect of treatment with AJM300 or anti-alpha 4 integrin mAb (PS/2) on the development of colitis.
Data represents mean (n=8).

Colon weight



T-cell infiltration into the lamina propria

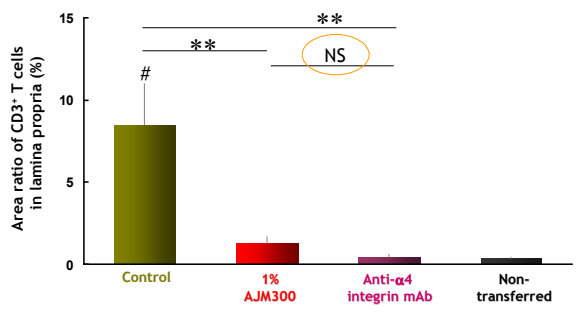


Figure 5.
Effect of oral treatment with AJM300 on the colon weight (left) and CD3⁺ T cell infiltration into the lamina propria (right).

Data represents mean±SE (n=8).
Colon Weight; ###p<0.001 vs Non-transferred by student's t test. ***p<0.01, NS : p≧0.05 by Tukey Kramer's test.
T cell; #p<0.05 vs Non-transferred by Welch's test. **p<0.01, NS : p≧0.05, by Steel-Dwass' test.

Occupancy

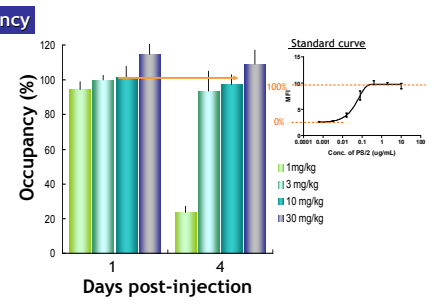


Figure 6. Alpha 4 integrin occupancy following i.p. administration of anti-alpha 4 integrin mAb (PS/2) in mice.
Data represents mean±SE(n=4 or 5).

The efficacy of 1% AJM300 was comparable to that of anti-alpha 4 integrin monoclonal antibody (PS/2) at the dose of 10 mg/kg, at which the saturation of alpha 4 integrin blockade was achieved for at least 4 days per dose.

Conclusions

- These results suggested that AJM300 prevented the development of experimental colitis induced by adoptive transfer of IL-10 deficient CD4⁺ T cells in mice.
- The efficacy which was accompanied by the reduction of T-cell infiltration was observed at 1% in the diet.
- The efficacy of AJM300 at 1% of diet was shown to be comparable to the maximum efficacy of alpha 4 integrin blockade.
- AJM300 might become the promising oral drug for the treatment of patients with IBD.

Presented at the GASTRO2009, Nov. 21-25, 2009: London, United Kingdom.