Bifidobacterium infantis 35624 modulates host inflammatory processes beyond the gut

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Abbreviations: *B. infantis*, *Bifidobacteria infantis*; UC, ulcerative colitis; CFS, chronic fatigue syndrome; TNF-α, tumor necrosis factor; IL, interleukin; PBMCs, peripheral blood mononuclear cells; LPS, lipopolysaccharide; PASI, psoriasis area severity index; CAIA, clinical activity index assessment; IBS, irritable bowel syndrome; IBD, inflammatory bowel disease

Certain therapeutic microbes, including *Bifidobacteria infantis* (*B. infantis*) 35624, exert beneficial immunoregulatory effects by mimicking commensal-immune interactions; however, the value of these effects in patients with non-gastrointestinal inflammatory conditions remains unclear. In this study, we assessed the impact of oral administration of *B. infantis* 35624 for 6–8 weeks on inflammatory biomarker and plasma cytokine levels in patients with ulcerative colitis (UC) (n = 22), chronic fatigue syndrome (CFS) (n = 48) and psoriasis (n = 26) in three separate randomized, double-blind, placebo-controlled interventions. Additionally, the effect of *B. infantis* 35624 on immunological biomarkers in healthy subjects (n = 22) was assessed. At baseline, both gastrointestinal (UC) and non-gastrointestinal (CFS and psoriasis) patients had significantly increased plasma levels of C-reactive protein (CRP) and the pro-inflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) compared with healthy volunteers. *B. infantis* 35624 feeding resulted in reduced plasma CRP levels in all three inflammatory disorders compared with placebo. Interestingly, plasma TNF-α was reduced in CFS and psoriasis while IL-6 was reduced in UC and CFS. Furthermore, in healthy subjects, LPS-stimulated TNF-α and IL-6 secretion by peripheral blood mononuclear cells (PBMCs) was significantly reduced in the *B. infantis* 35624-treated groups compared with placebo following eight weeks of feeding. These results demonstrate the ability of this microbe to reduce systemic pro-inflammatory biomarkers in both gastrointestinal and non-gastrointestinal conditions. In conclusion, these data show that the immunomodulatory effects of the microbiota in humans are not limited to the mucosal immune system but extend to the systemic immune system.

Introduction

There is persuasive evidence from several sources indicating that the gut microbiota has an influence on the development and maintenance, not only of the mucosal,[1,2] but also the systemic immune response.[3,4] This raises the therapeutic vista of modifying the systemic immune response by enteric microbes. For example, species as varied as *Clostridia*[5] and *Bifidobacteria*[6,7] have been shown to induce regulatory T cells which, theoretically, could modulate immune-inflammatory or autoimmune diseases. The organism *Bifidobacterium longum* subsp *infantis* (*B. infantis*) 35624 induces regulatory T cells in animal models with activity both in the gut and at extra-intestinal sites.[7-11] It has also been shown to increase the relative proportion of regulatory T cells in the peripheral blood of healthy human volunteers.[12] This raises the question as to whether regulatory T cell induction following administration of *B. infantis* 35624 would be sufficient to influence inflammatory mediator production in inflammatory disorders both in and beyond the gut in humans.

To address this, we studied cytokine profiles before and after administration of this organism to patients with ulcerative colitis (UC) and two extra-intestinal inflammatory diseases; psoriasis and chronic fatigue syndrome (CFS). The results show that *B. infantis* 35624-feeding significantly reduced plasma CRP and pro-inflammatory cytokine levels in both gastrointestinal and non-gastrointestinal inflammatory disorders.

Results

Baseline plasma pro-inflammatory biomarkers were elevated in UC and the extra-intestinal conditions psoriasis and CFS compared with healthy volunteers. CRP (p < 0.001), TNF-α (p < 0.001) and IL-6 (p < 0.05), were elevated in patients with psoriasis, CFS and UC compared with healthy volunteers (**Fig. 1**). In

Table 1. *B. infantis* 35624-feeding induces a reduction in absolute CRP levels in the majority of inflammatory disorder patients compared with placebo-fed patients

Disease indication	Treatment	Biomarker	Pre-treatment level (mg/L)	Post-treatment level (mg/L)
Chronic fatigue syndrome	*B. infantis*	CRP	2.35 (1.36–5.65)	2.07 (1.05–4.77)
	Placebo	CRP	1.92 (0.41–9.78)	3.02 (1.52–9.72)
Psoriasis	*B. infantis*	CRP	4.47 (3.59–7.97)	2.89 (2.02–5.36)
	Placebo	CRP	2.68 (1.33–4.33)	3.55 (2.46–5.79)
Ulcerative colitis	*B. infantis*	CRP	3.81 (1.67–10.66)	1.82 (0.83–15.18)
	Placebo	CRP	8.91 (1.18–50.94)	36.20 (3.26–86.14)

Results expressed as median (interquartile ranges).

Table 2. *B. infantis* 35624-feeding induces a reduction in absolute IL-6 and TNF-α levels in inflammatory disorder patients compared with placebo-fed patients

Disease Indication	Treatment	Cytokine	Pre-treatment level (pg/ml)	Post-treatment level (pg/ml)
Psoriasis	*B. infantis*	IL-6	1.62 (1.05–2.96)	1.48 (0.94–1.93)
	Placebo	IL-6	1.73 (1.32–2.78)	1.42 (1.12–2.47)
Chronic fatigue syndrome	*B. infantis*	IL-6	0.73 (0.60–0.96)	0.66 (0.50–0.85
	Placebo	IL-6	0.85 (0.70–1.21)	0.92 (0.83–1.27)
Ulcerative colitis	*B. infantis*	IL-6	2.17 (1.05–3.96)	1.98 (1.29–2.60)
	Placebo	IL-6	3.28 (1.05–4.95)	5.57 (1.39–7.96)
Psoriasis	*B. infantis*	TNF-α	8.20 (7.12–11.05)	7.67 (6.57–8.43)
	Placebo	TNF-α	7.06 (6.48–8.10)	7.40 (6.35–8.91)
Chronic fatigue syndrome	*B. infantis*	TNF-α	7.50 (6.42–7.85)	6.82 (5.75–9.22)
	Placebo	TNF-α	8.01 (6.07–8.90)	8.36 (6.88–9.84)
Ulcerative colitis	*B. infantis*	TNF-α	9.38 (7.49–9.84)	7.12 (8.77–9.84)
	Placebo	TNF-α	6.82 (5.75–9.22)	7.03 (5.48–9.18)

Results expressed as median (interquartile ranges).

general, UC patients displayed the highest CRP levels compared with healthy volunteers, while plasma TNF-α and IL-6 levels were comparable for the different disease states.

***B. infantis 35624* reduced plasma pro-inflammatory biomarkers in UC and extra-intestinal inflammatory conditions.** The administration of *B. infantis* 35624 was associated with a significant reduction in plasma pro-inflammatory biomarkers in patients with psoriasis, CFS and, to a lesser extent, in those with UC.

CRP. Plasma CRP levels were significantly reduced in *B. infantis* 35624-fed subjects compared with placebo controls in psoriasis ($p = 0.0425$), CFS ($p = 0.0285$) and UC patients ($p = 0.0327$). Data are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient (**Fig. 2**) with the median values (interquartile range) presented in **Table 1**.

When comparing pre-and post-feeding levels, plasma CRP was significantly reduced in psoriasis ($p = 0.0161$) and CFS ($p = 0.0393$) after eight weeks of feeding *B. infantis* 35624 but increased slightly in the placebo group after eight weeks of feeding (**Fig. 3A and B**). There was no statistically significant

decrease in CRP levels for UC patients following six weeks *B. infantis* 35624 feeding; however, CRP levels in the placebo group increased post treatment, likely due to these patients not receiving steroid treatment during the trial period (**Fig. 3C**).

TNF-α. Plasma TNF-α levels were significantly reduced in *B. infantis* 35624-fed subjects compared with placebo controls in psoriasis ($p = 0.0405$) and CFS ($p = 0.0214$). However, no significant difference in the levels of TNF-α was observed between *B. infantis* 35624-fed UC patients compared with placebo following six weeks feeding. Data are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient (**Fig. 4**) with the median values (interquartile range) presented in **Table 2**.

When comparing pre- and post-feeding levels, plasma TNF-α was significantly reduced in psoriasis ($p = 0.0269$) and CFS ($p = 0.0129$) after 8 wk of feeding with *B. infantis* 35624 but increased slightly in the placebo group (**Fig. 5A and B**). In UC patients, there was no statistically significant decrease in TNF-α levels following six weeks *B. infantis* 35624 feeding; however, TNF-α

Figure 1 (See opposite page). Plasma pro-inflammatory biomarkers are elevated in patients with inflammatory disorders. Plasma CRP and pro-inflammatory cytokines levels are significantly elevated in chronic fatigue syndrome (CFS) (n = 48), psoriasis (n = 26) and ulcerative colitis (UC) (n = 22) patients compared with healthy volunteers (n = 35). Results are expressed as median values. (Healthy volunteers vs. each inflammatory disorder) (Mann-Whitney U test) *p < 0.05, **p < 0.01, ***p < 0.01.

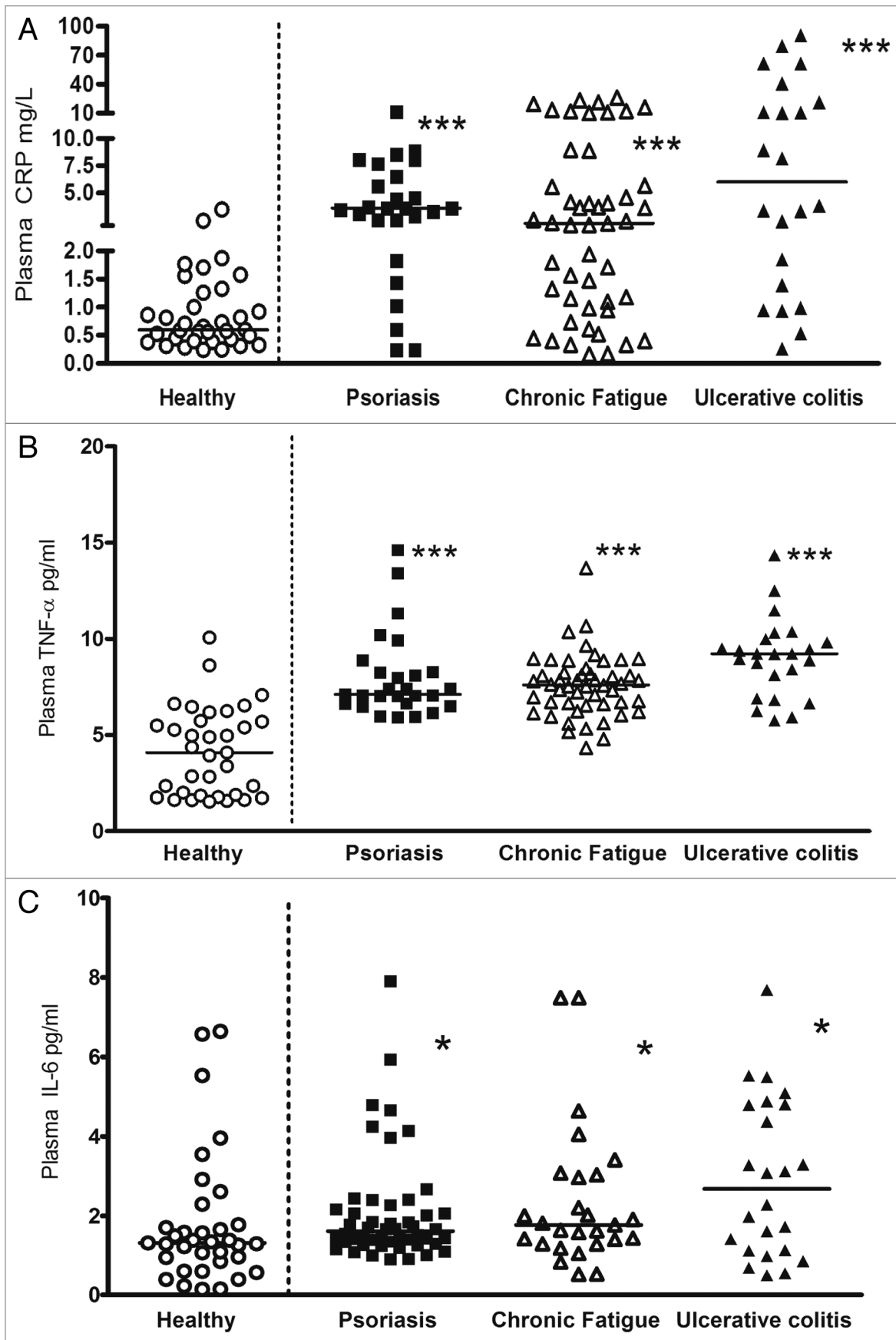**Figure 1.** For figure legend, see page 326.

Table 3. In comparison to placebo, *B. infantis* 35624 feeding reduces plasma CRP, TNF-α and IL-6 levels, expressed as % change from baseline, in inflammatory disorder patients

Disease Indication	Treatment	(%) Change in the level of CRP	(%) Change in the level of TNF-α	(%) Change in the level of IL-6
Psoriasis	*B. infantis*	−34.0 (−64.0, −7.25)	−15.5 (−20.0, 3.50)	−3.0 (−13.0, 16.0)
	Placebo	2.0 (−31.75,154.8)	0.0 (−22.0, 15.0)	−5.0 (−20.5, 11.25)
Chronic fatigue syndrome	*B. infantis*	−12.0 (−55.0, 15.75)	−6.0 (−13.0, 4.0)	−14.0 (−26.5, −5.0)
	Placebo	34.0 (−15.0, 211.3)	3.0 (−6.0, 17.0)	4.0 (−10.5, 25.5)
Ulcerative colitis	*B. infantis*	−38.0 (−79.0, 178.0)	−5.0 (−9.50, 8.50)	−5.0 (−33.0, 36.5)
	Placebo	261.0 (19.0, 395.5)	5.0 (−11.0, 20.0)	27.0 (−3.5, 137.0)

Table 4. More patients with inflammatory disorders achieve reductions in plasma CRP, TNF-α and IL-6 levels following feeding with *B. infantis* 35624 compared with placebo-fed patients

Disease indication	Treatment	Number of subjects ↓IL-6, ↓TNF-α, ↓CRP	Total number of subjects	Percent of Subjects ↓IL-6, ↓TNF-α, ↓CRP
Psoriasis	*B. infantis*	9	12	75%
	Placebo	1	14	7%
Chronic fatigue syndrome	*B. infantis*	20	28	71%
	Placebo	3	20	11%
Ulcerative colitis	*B. infantis*	8	13	62%
	Placebo	0	9	0%
Inflammatory diseases	*B. infantis*	37	53	70%
	Placebo	4	43	9%

levels in the placebo group increased from pretreatment (**Fig. 5C**).

IL-6. Plasma IL-6 levels were numerically lower in *B. infantis* 35624-fed patients compared with placebo controls in CFS (p = 0.054) and UC (p = 0.057) but remained unchanged in psoriasis. Data are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient (**Fig. 6**) with the median values (interquartile range) presented in **Table 2**.

When comparing pre-and post-feeding for CFS patients, plasma IL-6 levels were significantly reduced (p = 0.0021) after eight weeks of feeding *B. infantis* 35624 but remained unchanged in the placebo group (**Fig. 7B**). There was no statistically significant decrease in plasma IL-6 in psoriasis and UC following 6–8 sk feeding *B. infantis* 35624 compared with pretreatment levels. However, while plasma IL-6 levels remained unchanged in the psoriasis placebo group, they increased in the UC placebo-fed group (**Fig. 7A and C**).

Modifications of plasma cytokine and CRP levels are correlated with *B. infantis* feeding. To further explore relationships with *B. infantis* 35624 feeding and responses in the various inflammatory markers, individual patient changes from baseline values of CRP, TNF-α and IL-6 were plotted in 3D scatter plots for each inflammatory disease; psoriasis, chronic fatigue syndrome and ulcerative colitis. This analysis revealed a separation of *B. infantis* 35624-fed patients from placebo-fed patients in all three inflammatory diseases (**Fig. 8**). To demonstrate a unique responder biomarker pattern, the individual CRP, TNF-α and IL-6 as represented by percentage change from baseline in the inflammatory disorder patients compared with placebo-fed patients (**Table 3**). At week 6–8, 75% of psoriasis patients, 71% of CFS patients and 62% of UC patients displayed decreased levels of CRP, TNF-α and Il-6, when fed *B. infantis* 35624. In contrast, only 7% of psoriasis patients, 11% of CFS patients and 0% of the UC patients displayed decreased levels of CRP, TNF-α and IL-6 in the placebo-fed group (**Table 4**). Furthermore, 70% of all patients fed *B. infantis* 35624, independent of the nature of their inflammatory disorder, demonstrated a reduction in CRP, TNF-α and IL-6 compared with 9% in the placebo group (**Table 4**).

***B. infantis* 35624 reduces pro-inflammatory biomarkers in healthy subjects.** In contrast to the inflammatory disease patients described above, the plasma levels of CRP, TNF-α and IL-6 in healthy human volunteers were unaffected by eight weeks feeding with *B. infantis* 35624 (data not shown). However, *B. infantis* 35624 feeding for eight weeks resulted in a reduction in the in vitro secretion of TNF-α (p < 0.05) and IL-6 (p < 0.05) from lipopolysacharide-(LPS) stimulated peripheral blood mononuclear cells (PBMCs) in comparison to those fed placebo (**Fig. 9**).

Discussion

Our findings show that oral administration of *B. infantis* 35624 modulates the cytokine milieu across both gastrointestinal and non-gastrointestinal inflammatory disorders and healthy subjects. *B. infantis* 35624 significantly reduced plasma CRP levels in all patient groups, plasma TNF-α in the non-gastrointestinal disorders, psoriasis and CFS, while non-statistically significant trends toward a reduction in levels of IL-6 were seen in patients with CFS and UC. Furthermore, *B. infantis* 35624 altered responses to inflammatory stimuli in ex vivo cultures from healthy volunteers.

Chronic inflammatory diseases such as UC, CFS and psoriasis are characterized by an over-production of pro-inflammatory
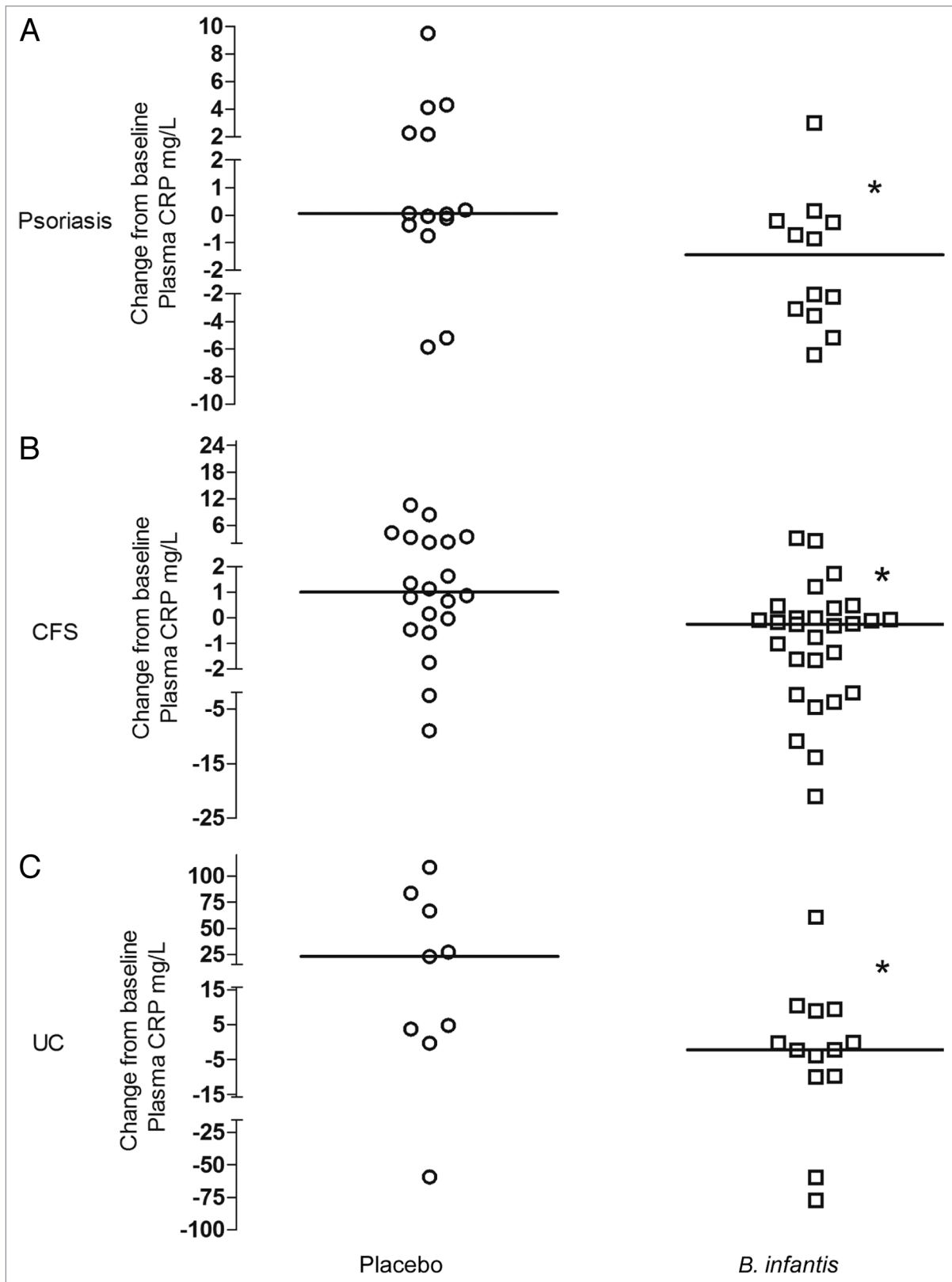
Figure 2. *B. infantis* 35624-feeding reduces plasma CRP levels compared with placebo. Plasma levels of CRP were significantly reduced in the *B. infantis* 35624 treated groups compared with placebo treatment following 6–8 wk of feeding in patients with chronic fatigue syndrome (CFS), psoriasis and ulcerative colitis (UC). Results are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient. *p < 0.05 vs. placebo; (Mann-Whitney U test).
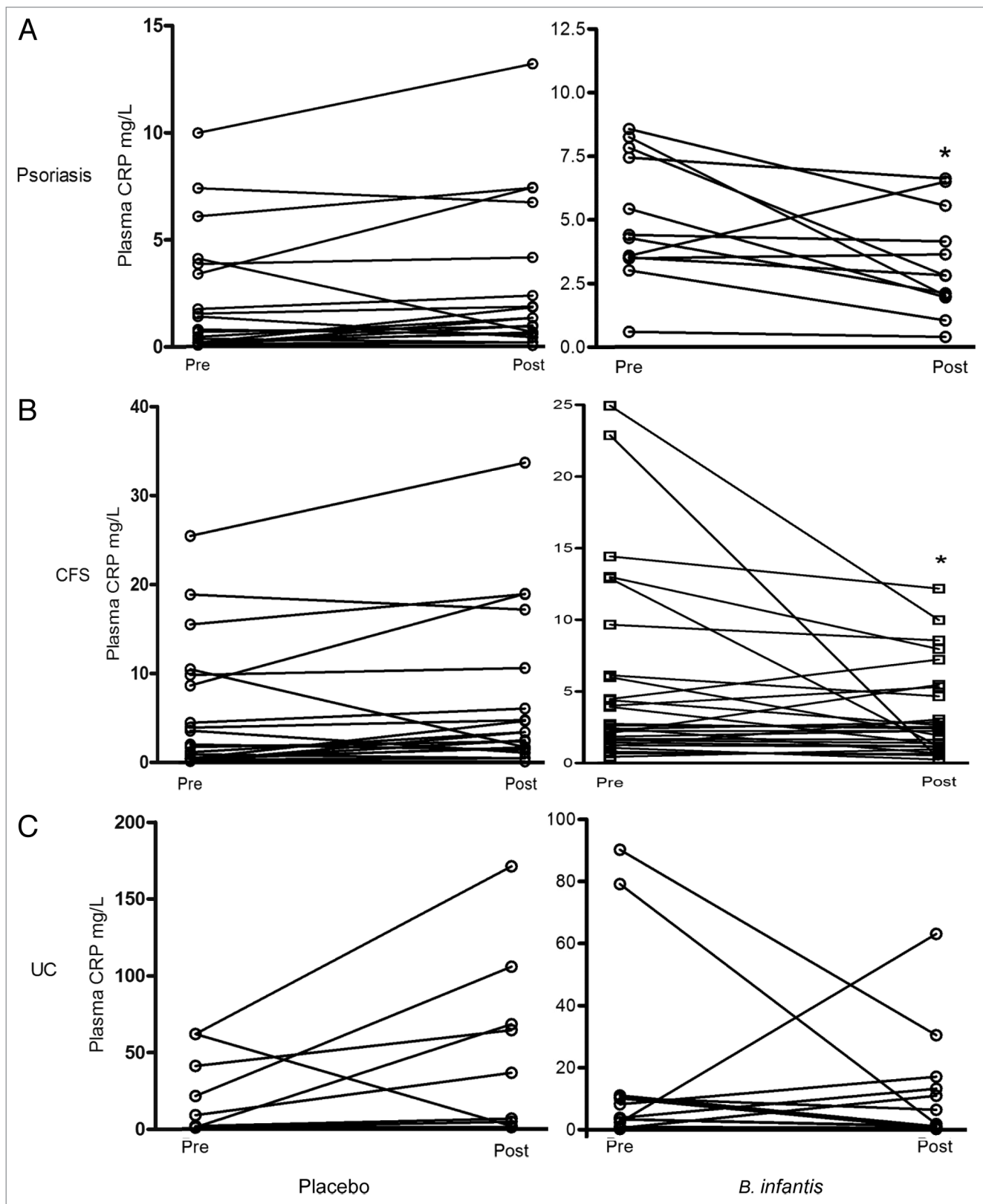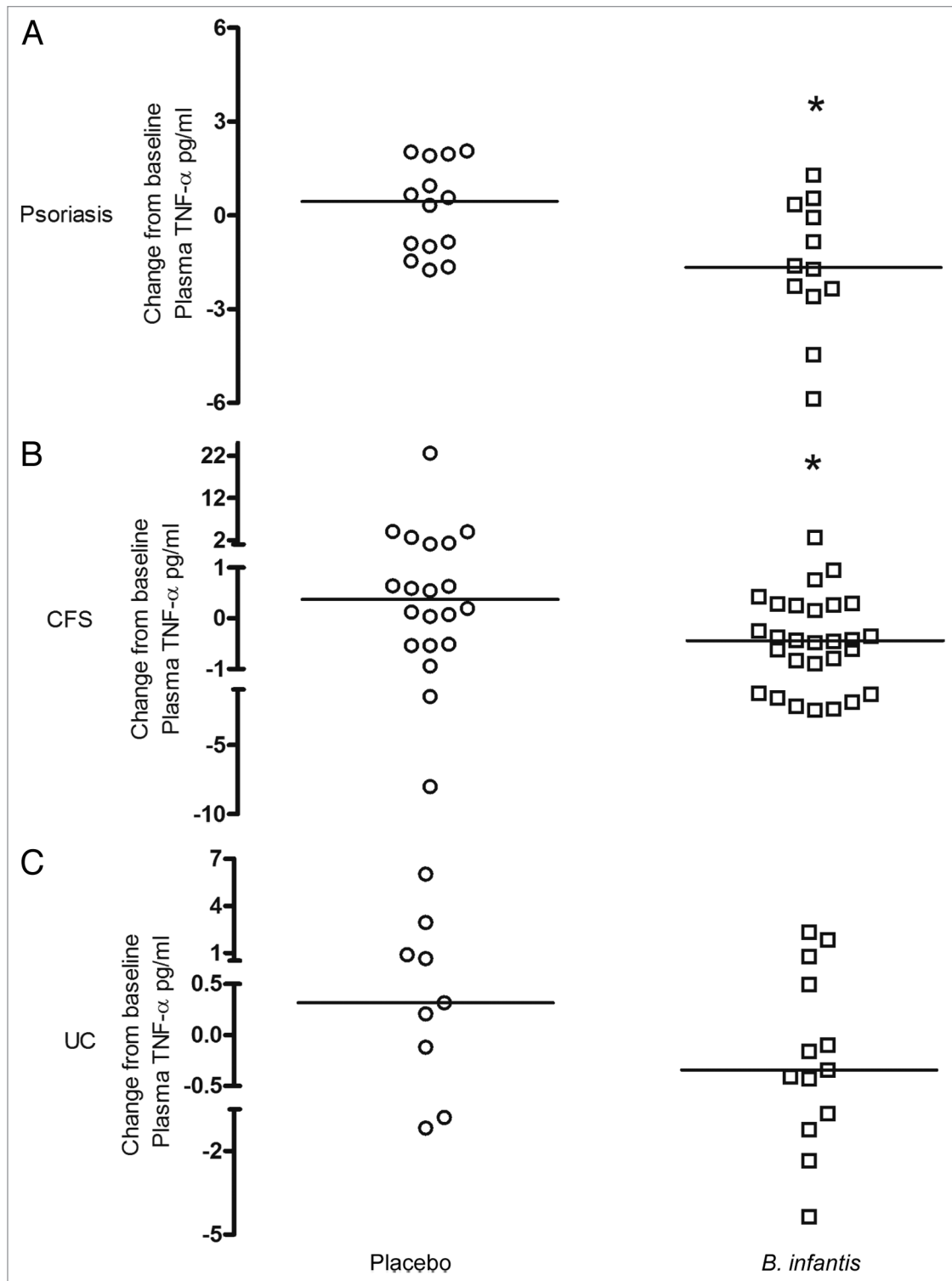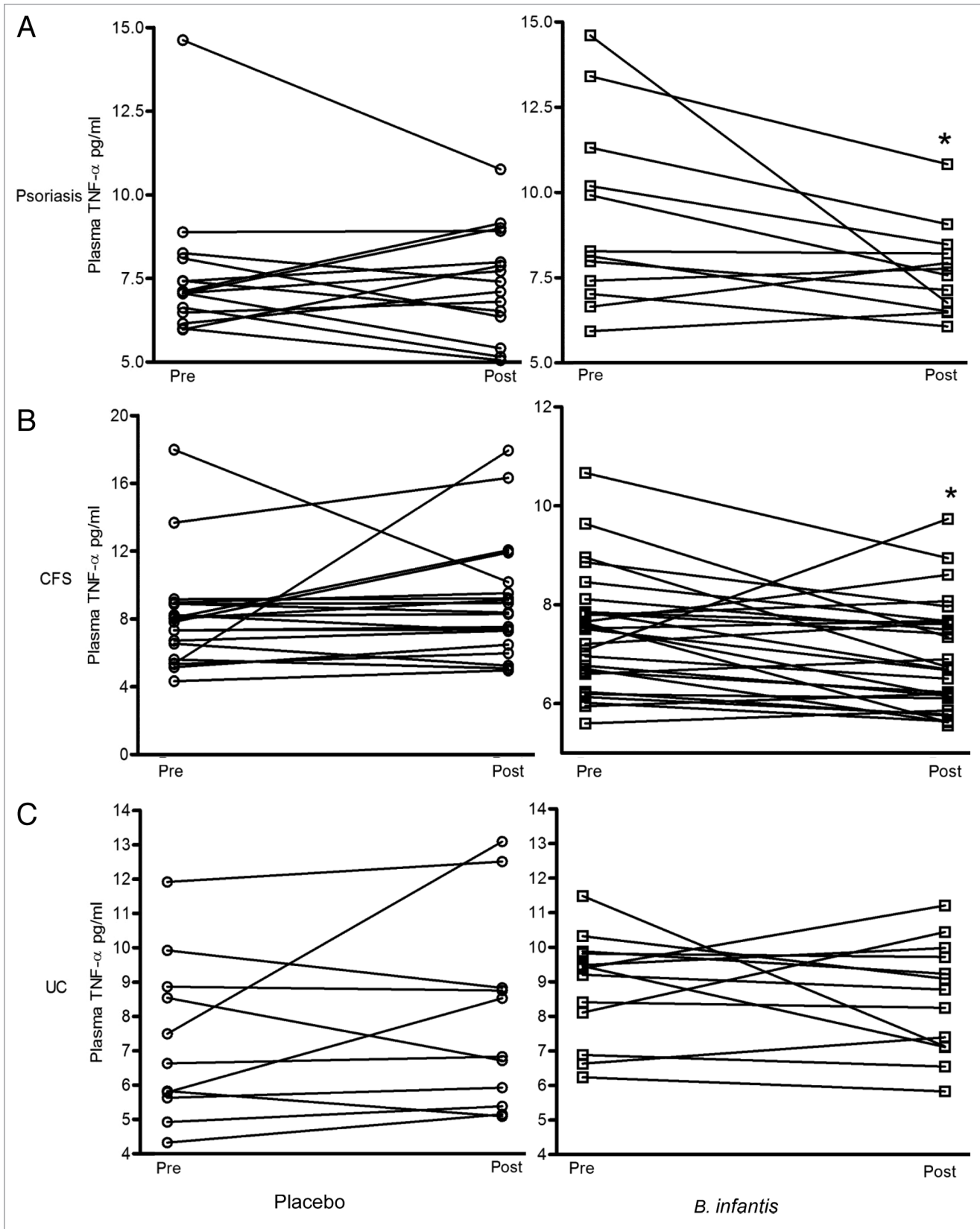
Figure 3. *B. infantis* 35624-feeding reduces plasma CRP levels compared with pretreatment level. Placebo-feeding for 8 weeks did not alter plasma CRP levels compared with pretreatment levels in any patients with inflammatory disorders. Plasma CRP levels were significantly reduced following 8 wk of feeding with *B. infantis* 35624 compared with pretreatment levels in (**A**) psoriasis (n = 12) and (**B**) chronic fatigue syndrome (CFS) (n = 28), However, there was no difference in CRP levels following 6 wk feeding with *B. infantis* 35624 in patients with (**C**) ulcerative colitis (UC) (n = 13) compared with their pretreatment levels. Results are expressed as the individual responses of each patient (mg/L) (pre-treatment vs. post-treatment level) *p < 0.05 (Wilcoxon matched pair test).

Figure 4. *B. infantis* 35624-feeding reduces plasma TNF-α levels compared with placebo. Plasma levels of TNF-α were significantly reduced in the *B. infantis* 35624-treated groups compared with placebo treatment following 8 wk of feeding in patients with chronic fatigue syndrome (CFS) and psoriasis. However, there was no difference in TNF-α levels following 6 wk feeding with *B. infantis* 35624 in patients with ulcerative colitis (UC) compared with placebo treatment. Results are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient. *p < 0.05 vs. placebo; (Mann-Whitney U test).

Figure 5. For figure legend, see page 333.

cytokines. C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes and adipocytes in response to increased peripheral pro-inflammatory cytokines, such as TNF-α and IL-6,[13,14] and the serum or plasma level is a useful and clinically relevant indicator of systemic pro-inflammatory activity in multiple inflammatory states.[15] In this study, plasma CRP was significantly elevated in all conditions investigated compared with healthy controls. These elevated levels of CRP concur with the results of previous studies conducted in CFS,[16,17] psoriasis,[18-21] and UC patients.[22] In standard clinical laboratories the median value of plasma CRP for healthy subjects is < 1 mg/L.[23-25] Ultrasensitive assays have associated low-grade inflammation (CRP > 2.4 mg/L) with increased risk for coronary artery disease,[26] while levels of CRP from 10–1000 mg/L are associated with overt inflammatory and infectious disorders.[25] Interestingly, *B. infantis* 35624 significantly reduced plasma CRP levels in all three inflammatory conditions examined. Regarding the significance of changes in CRP following treatment, the best illustration is provided by studies in heart disease where very small increments in CRP (1–2 mg/L) were associated with substantial increases in risk for coronary events.[27]

The effect of other microbes on CRP levels has been quite variable. Studies have shown that microbial treatment reduced CRP levels in the serum or plasma in ulcerative colitis[28-30] while others noted an increase in serum CRP levels in a non-gastrointestinal condition eczema dermatitis[31,32] or no effect in both gastrointestinal[33] and non-gastrointestinal conditions.[31,34,35] This suggests that not all commensal microbes can induce this effect in humans.

TNF-α and IL-6 are pro-inflammatory cytokines which are elevated in a variety of inflammatory conditions and involved in transcriptional regulation of CRP.[25,36] They are not employed in clinical practice but both of these cytokines have been targeted by aggressive anti-cytokine biologic agent therapy in the treatment of autoimmune diseases.[37,38] The attenuation of CRP following *B. infantis* 35624 treatment in this study is consistent with the reduction in circulating levels of both of those pro-inflammatory cytokines. In general, reduction in inflammatory markers, such as those seen in this study would be regarded as indicative of clinical remission and of a lower risk of relapse.[22,39,40]

Patients with CFS, psoriasis and UC had higher baseline TNF-α levels compared with healthy controls. These findings are in agreement with other studies.[22,41-43] Following 8 wk of treatment with *B. infantis* 35624, a significant attenuation of TNF-α was observed for CFS and psoriasis patients; no such effect was noted with placebo treatment. A trend toward decreased TNF-α in the UC group was also observed, but this did not reach statistical significance, perhaps due to the shorter treatment time (i.e., 6 wk). These data implicate TNF-α in the pathophysiology of inflammatory conditions and support the use of specific and well selected therapeutic microbes in alleviating the inflammatory component of such conditions, despite the often conflicting literature.[34,35,44-47]

Plasma IL-6 levels were significantly higher in CFS, psoriasis and UC patients compared with healthy controls, which is consistent with the findings of previous studies in psoriasis,[42,48] CFS[43,49] and UC.[50] Though previous studies have indicated that certain therapeutic microbes could significantly reduce IL-6 levels in both patients with gastrointestinal inflammatory disorders and healthy controls,[44,51-53] plasma IL-6 levels were only marginally decreased following *B. infantis* 35624 administration in this study. These data reinforce the hypothesis that individual elements of the microbiota influence specific components of the host immune response and not all members of the microbiota, or even not all members of the same species, exert an identical effect.

Overall, 70% of all of the individuals with UC, CFS or psoriasis fed *B. infantis* 35624 for 6–8 wk showed marked decreases in all three biomarkers. Despite the limitations of post hoc analysis, this finding supports the concept of *B. infantis* 35624 acting centrally, perhaps through increased numbers of regulatory T cells, to minimize pro-inflammatory activity in vivo. Furthermore, in the ex vivo studies, secretion of IL-6 and TNF-α from LPS-stimulated peripheral blood mononuclear cells (PBMCs) was significantly reduced by *B. infantis* 35624 feeding in healthy controls compared with placebo-fed controls.

There are limitations to this study: first, the n value for each clinical condition studied is relatively low; collectively, however, the message is consistent. While the numbers included were sufficient to demonstrate statistical significance for objectively measured biomarkers, larger patient populations would be required to demonstrate clinical efficacy. Second, only one daily dose of this microbe was studied and a dose-dependent anti-inflammatory effect was, therefore, not demonstrated. It is noteworthy in this respect, that many naturally occurring and even biologic agents, such as anti-TNF-α strategies, do not exhibit clear dose-response profiles.

In conclusion, oral administration of a single microbial agent, *B. infantis* 35624, was sufficient to reduce systemic inflammatory biomarkers in both gastrointestinal and extra-intestinal inflammatory disorders. This is consistent with the hypothesis that certain elements of the enteric microbiota can induce mucosal immunoregulatory responses that can exert an effect systemically.

Methods

Psoriasis patients. Twenty-six male and female patients aged between 18 and 60 y with mild to moderate chronic plaque psoriasis with a psoriasis area severity index (PASI) < 16 were
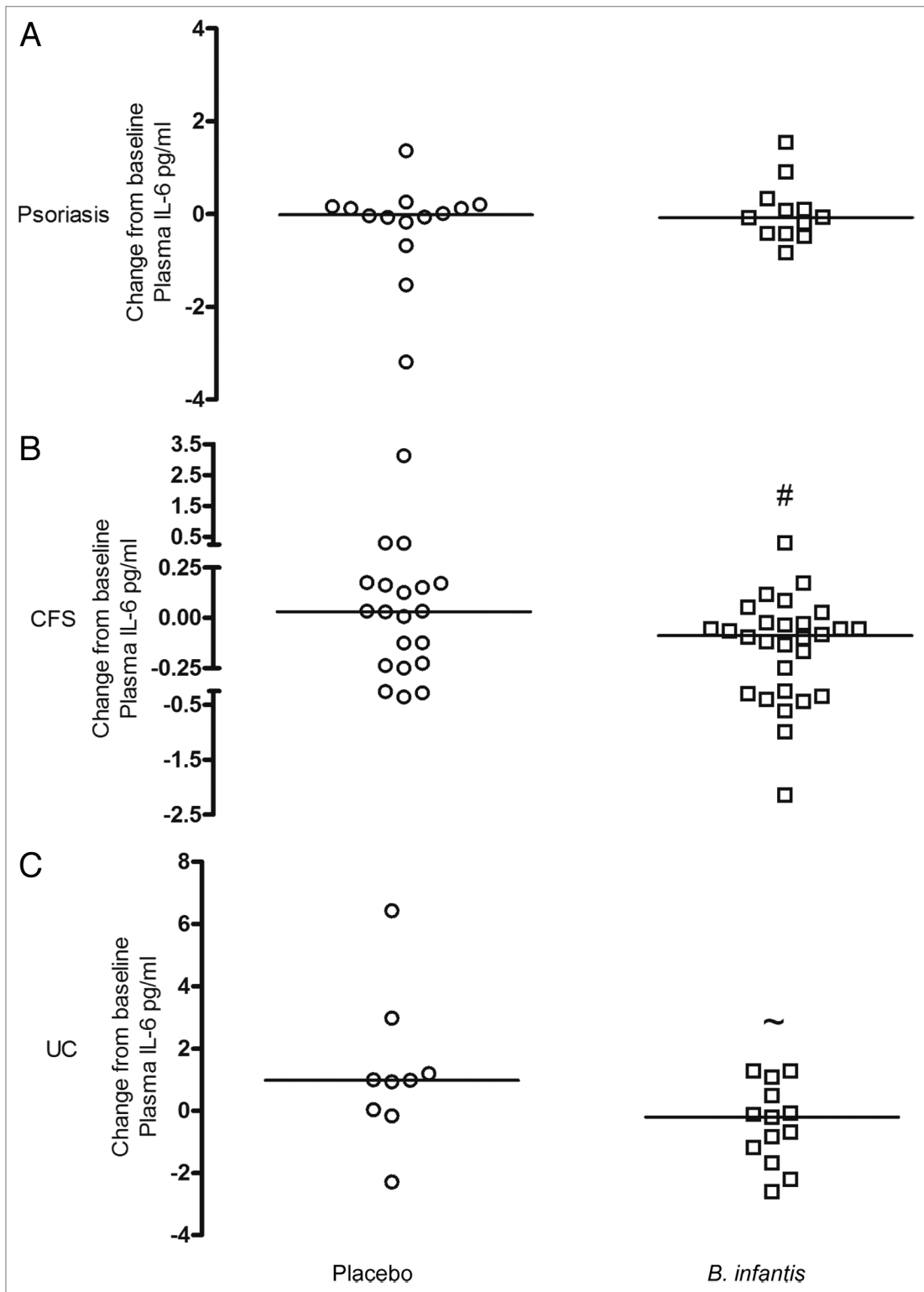
Figure 6. *B. infantis* 35624-feeding reduces plasma IL-6 levels compared with placebo. Plasma levels of IL-6 were reduced in the *B. infantis* 35624 treated groups compared with placebo treatment following 6–8 wk of feeding in patients with chronic fatigue syndrome (CFS) (#p = 0.054) and ulcerative colitis (UC) (~p = 0.057). However, there was no difference in IL-6 levels following 8 wk feeding with *B. infantis* 35624 in patients with psoriasis compared with placebo treatment. Results are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient. *p < 0.05 vs. placebo groups (Mann-Whitney U test).
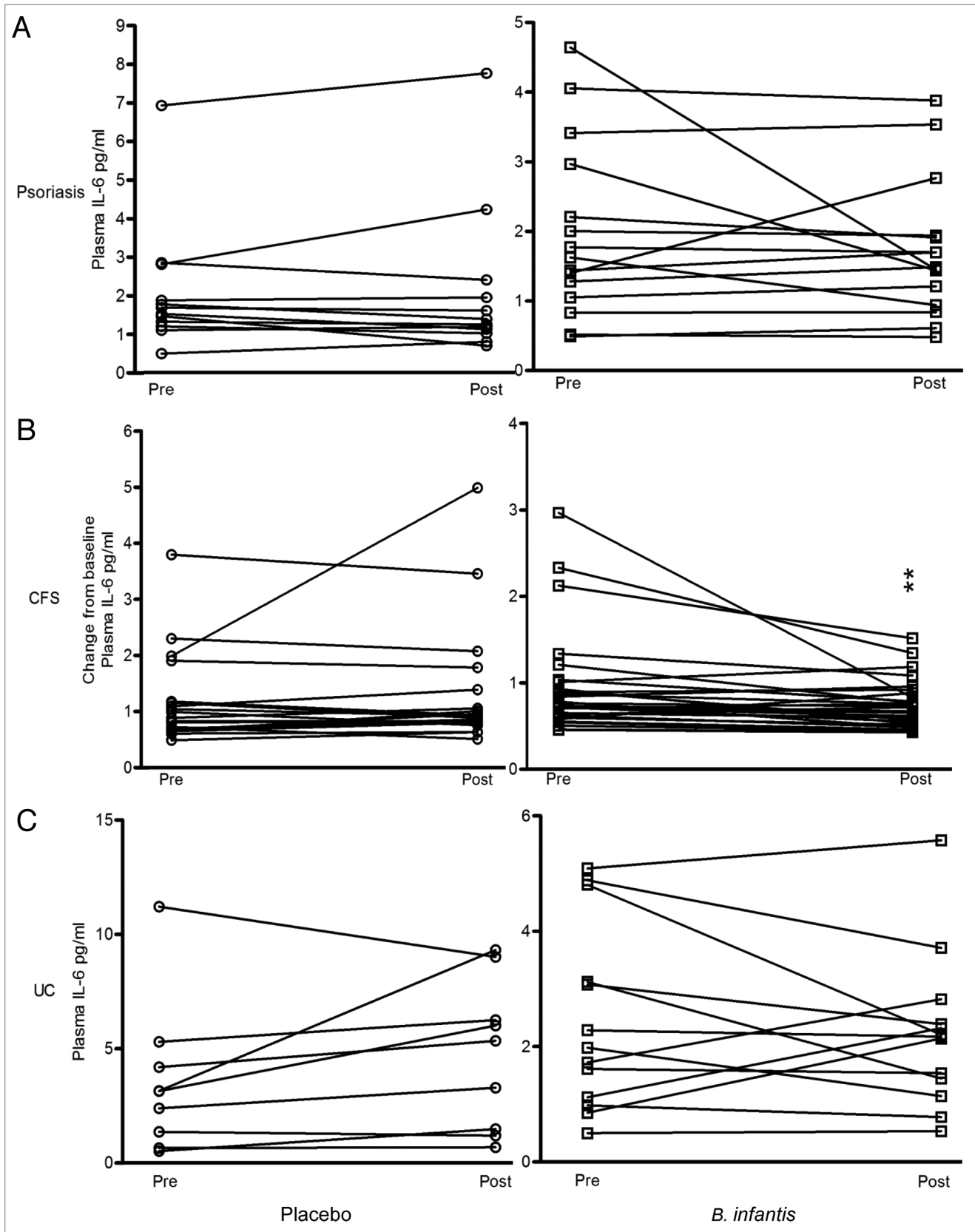
Figure 7. For figure legend, see page 336.

identified from the community by means of local advertising in newspapers and general practice clinics.

Ulcerative colitis patients. Twenty-two male and female patients aged between 18 and 75 y with mild to moderate active ulcerative colitis (UC) with a clinical activity index assessment (CAIA) score ≥ 3 were identified. Subjects were recruited from the inflammatory bowel disease (IBD) clinic at Cork University Hospital. These subjects had a clinical diagnosis of UC confirmed by clinical, radiological and/or pathological evidence, and their level of disease activity required a change in treatment. All these patients were on the optimal dose of mesalazine (5-ASA) and remained on the same dose throughout the study.

Chronic fatigue syndrome patients. Patients with chronic fatigue syndrome (CFS) meeting the criteria outlined by the Centers for Disease Control (CDC) were recruited from gastroenterology and rheumatology clinics at Cork University Hospital. A total of 48 female patients between 18 and 65 y of age were selected. Those who had other diseases of the gastrointestinal tract, including inflammatory bowel disease (IBD) and clinically significant systemic diseases, individuals diagnosed with lactose intolerance or immunodeficiency, individuals who had undergone any abdominal surgery (with the exception of hernia repair and appendectomy), and those with a psychiatric illness were excluded. The diagnosis of CFS was made using the 1994 Centers for Disease Control and CFS International Study Group diagnostic criteria.[54] The international criteria are based on the fulfillment of two major criteria: CFS causing incapacity, lasting more than 6 mo, and the exclusion of associated medical and psychiatric conditions, as well as the concurrence of a series of symptom-based criteria, particularly rheumatologic and neuropsychological symptomatology.

Healthy subject population. Thirty-five healthy volunteers (25 female) aged between 18 and 65 were recruited by direct advertisement on the university campus and in a local newspaper. These subjects had no evidence of gastrointestinal tract disease, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), and were also free of any clinically significant systemic diseases including psoriasis and chronic fatigue syndrome. These 35 subjects were used as baseline references for the patients with inflammatory conditions. Twenty-two of the healthy adults were randomized to receive *Bifidobacterium infantis* 35264 (n = 10) or placebo (n = 12) for eight weeks.

Exclusion criteria. Pregnant or breast feeding females, individuals diagnosed with lactose intolerance or immunodeficiency, individuals who had undergone any abdominal surgery (with the exception of hernia repair and appendectomy) and those with a psychiatric illness or with significant hepatic, renal disease were excluded from all arms of the study, as were patients receiving immunosuppressant therapy or probiotics.

Each potentially eligible patient was evaluated by a full review of clinical history, physical examination, full blood count and routine biochemistry analysis. Subjects with any clinically significant abnormalities in any of these tests were excluded from the study.

Study design. The psoriasis arm of the study was performed in winter and early spring to minimize the confounding effect of ambient UV light; all the other arms of the trial were staggered throughout all seasons of the year. All interventions were performed as randomized, double-blind placebo-controlled studies.

Patients attended for baseline assessment of disease severity and blood was obtained for standard laboratory assessments of full blood count, electrolytes, renal and hepatic function hematology and biochemistry.

Nine ml of whole blood was collected in potassium EDTA tubes for biomarker analysis. Samples were centrifuged immediately and plasma frozen at –80°C. Measurements of plasma C-reactive protein (CRP), IL-6 and TNF-α were performed using an electrochemiluminescence multiplex system Sector 2400 Imager from Meso Scale Discovery where antibodies labeled with Sulfo-tag reagents emitted light upon electrochemical stimulation. This is an ultra-sensitive method which has a detection limit for CRP of 0.7 ng/ml, IL-6 of 0.3 pg/ml and TNF-α of 0.3 pg/ml.

Peripheral blood mononuclear cells (PBMCs) were isolated on day -1 (pre-feeding) and day 56 (post-feeding) from healthy volunteers. PBMCs (1×10^6/mL) were stimulated with 1 μg/ml lipopolysaccharide (LPS) for 72 h. PBMC supernatants were stored frozen at –80°C and analyzed for cytokine levels simultaneously using the Meso Scale Discovery multiplex platform.

Treatments. Each patient and 22 healthy controls received sachets containing either 1×10^{10} CFU viable *Bifidobacterium infantis* 35264 or 5 g Maltodextran as placebo per day. Probiotic and placebo were identically packaged. Both patients and investigators were blinded with regard to which treatment was being administered. CFS, psoriasis and healthy controls received either *Bifidobacterium infantis* 35264 or placebo for eight weeks; UC patients for six weeks.

Statistical analysis. Statistical analysis of cytokine and C-reactive protein levels was undertaken using GraphPad Prism for Windows (Version 5.0). Prior to analysis and to any comparisons being performed, data were examined for normality and homogeneity of variance using the D'Agostino and Pearson omnibus normality test. As there was evidence that the data were not normally distributed,non-parametric statistical methods were, therefore, used in the analysis of the data. When comparing the values for healthy controls vs. inflammatory diseases a Mann-Whitney U test was employed. To compare pre- vs. post-treatment cytokine levels in both *B. infantis* 35624 and placebo-fed patients a Wilcoxon signed rank test was used. When comparing
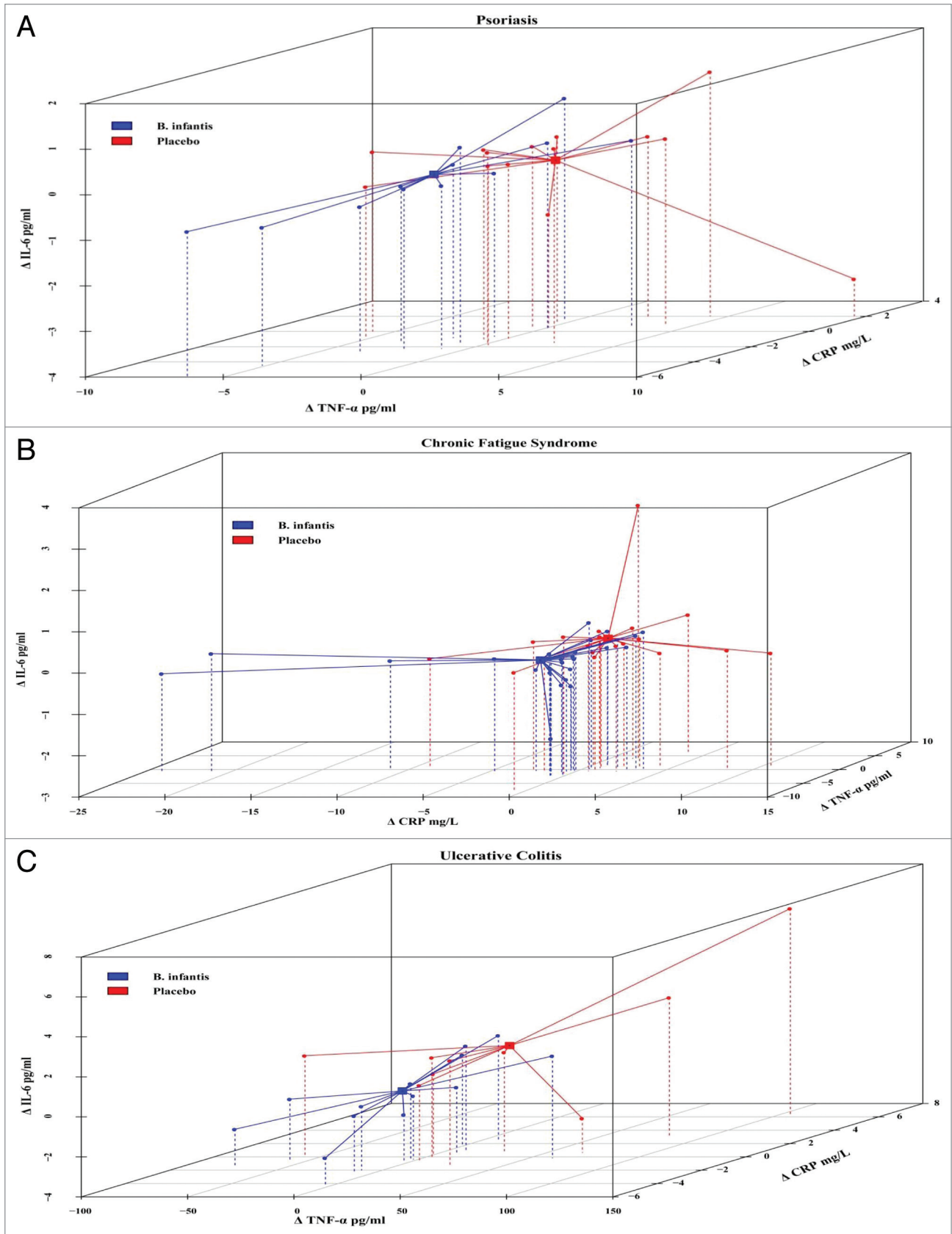
Figure 8. *B. infantis* 35624-feeding reduces pro-inflammatory biomarkers in a highly correlated way in inflammatory disorder patients. 3D Cluster analysis of plasma levels of CRP (z), TNF-α (x) and IL-6 (y) from patients with psoriasis and ulcerative colitis (UC) and of CRP (x), IL-6 (y) and TNF-α (z) from patients with chronic fatigue syndrome (CFS) displayed a separation between the *B. infantis* 35624 treated groups compared with the placebo treated groups. Results are expressed as the change from baseline (post-treatment minus pre-treatment level) for each patient.

the effects of *B. infantis* 35624 feeding and placebo on a patient's cytokine levels, differences were calculated for each patient as change from baseline (visit 2, week 0) to the end of feeding. The Mann-Whitney U test was then used to compare these changes from baseline in cytokine levels between *B. infantis* 35624 and placebo-fed subjects in all arms of the study. Differences were considered significant at $p < 0.05$.

Figure 9. *B. infantis* 35624-feeding reduces pro-inflammatory cytokines levels from LPS stimulated PBMCs in healthy volunteers. In vitro LPS stimulated PBMCs displayed decreased secretion of IL-6 and TNF-α following *B. infantis* 35624 oral consumption (n = 10) for 8 wk. This finding was not observed in the placebo group (n = 12). Results are expressed as the change from baseline (post-treatment minus pre-treatment level) for each individual. *p < 0.05 vs. placebo group (Mann-Whitney U test).

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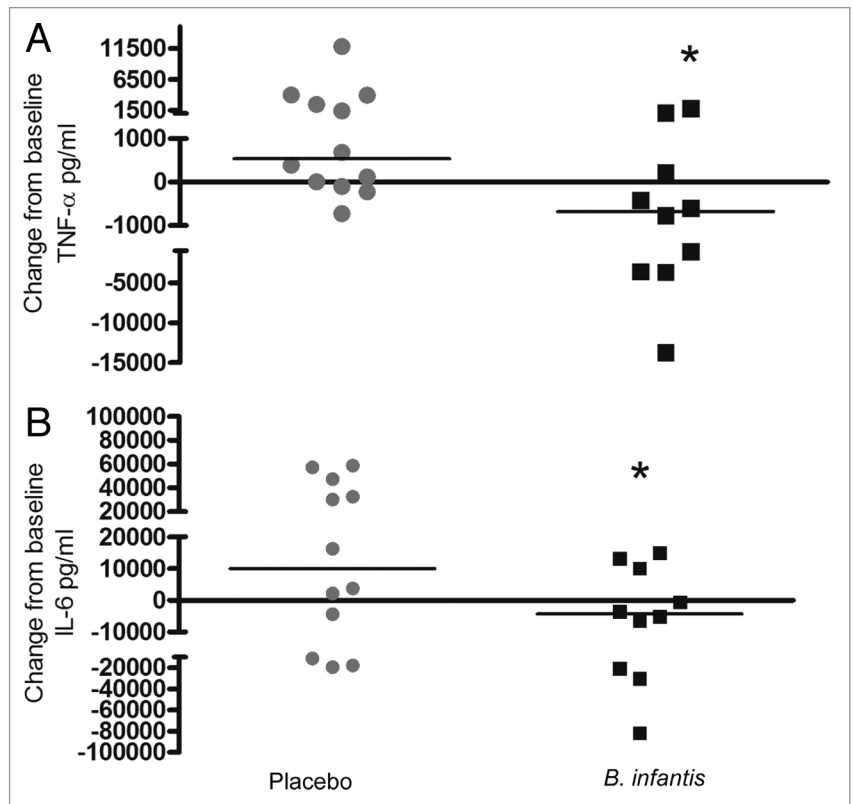
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