● DIFFERENTIAL EXPRESSION OF CELL ADHESION MOLECULES IN CROHN'S AND ULCERATIVE COLITIS. P.A.Dean, P.S.Ramsey, H.Nelson, Mayo Clinic, Rochester, MN.

The pathologic changes characterisitic of IBD are dependent upon migration of leukocytes into intestinal tissues. Although the migration of leukocytes to sites of inflammation is likely regulated by inducible cell adhesion molecules (CAMs) on vascular endothelium, little has been done to characterize the expression of these molecules in Crohn's and ulcerative colitis (CUC). **AIM**: To investigate the vascular expression of intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1) in Crohn's and CUC. **METHODS**: Immunohistochemistry was performed on frozen section tissues from normal colons (n=30) and from colons involved with Crohn's (n=15) and CUC (n=15). Microvessels were detected using the endothelial marker UEA1. The vascular expression of CAMs was detected using anti-ICAM-1 and anti-ELAM-1 monoclonal antibodies. The average number of UEA1 and ICAM-1 positive vessels (>5um) in the bowel wall was quantitated at 200x optical field by two independent observers. The vascular expression of ELAM-1 within the mucosa, submucosa, and muscularis was qualitatively graded. **RESULTS**: The average number of vessels staining for ICAM-1 was elevated in CUC (28 ± 18) compared to normal (2.7 ± 1.3)($p<0.00001$). The number of ICAM-1 positive vessels was also increased in Crohn's (53 ± 21) compared to normal (2.7 ± 1.3)($p<0.00001$) and compared to CUC (28 ± 18)($p<0.002$). The total number of microvessels (UEA1 positive) was not increased in Crohn's (66 ± 22) or CUC (99 ± 42) compared to normal bowel (86 ± 31). ELAM-1 expression was absent in normal colons, and differentially expressed in colitis with greater staining of mucosal vessels in CUC than Crohn's ($p<0.002$). **CONCLUSIONS**: A dramatic increase in the vascular expression of ICAM-1 and ELAM-1 was noted in both Crohn's and CUC despite no increase in the total number of vessels. Differential expression of both ICAM-1 and ELAM-1 was noted between Crohn's and CUC, with CAM expression in CUC focused more in the mucosa than in the muscularis. The enhanced expression of these molecules and their potential role in leukocyte trafficking supports the concept of CAM blocking therapy for the control of active IBD.

● THE VACUOLATING CYTOTOXIN OF HELICOBACTER PYLORI: COMPARING ISOLATES FROM DUODENAL ULCER AND NON-ULCER DYSPEPSIA PATIENTS. A.J. DeCross, L.J. Barrett, R.L. Guerrant, B.J. Marshall, University of Virginia, Charlottesville, VA.

The vacuolating cytotoxin of Helicobacter pylori (Hp) is a virulence factor of uncertain clinical significance. Our purpose is to compare the presence of vacuolating cytotoxin among Hp isolates from duodenal ulcer patients (DU's) and non-ulcer dyspepsia patients (NUD's).

Methods: 16 Hp isolates from DU's (non-steroidal medication use excluded) and 14 Hp isolates from NUD's were studied. The two groups were closely matched for patient age, race, and sex. Isolates were initially obtained from antral biopsies cultured on horse blood agar plates at 37°C in a 10% CO_2 incubator; they were then frozen in T-soy broth (15% glycerol) at -70°C. Frozen isolates were later recovered by reculturing on horse blood agar plates as above, and then inoculated from the blood agar plate into 10cc of brucella broth (10% fetal calf serum). The broth was cultured in a 50cc Erlenmeyer flask (loose cap) on a Gyrotory shaking platform (150 rpm) within the 37°C 10% CO_2 incubator. After 48 hours, the broth was centrifuged and the supernatant filtered. Chinese-Hamster-Ovary-K1 cultured cells were incubated overnight before exposure to filtered Hp broth supernatant for 48 hrs. The cells were Giemsa stained and assessed for the presence of intracellular vacuoles, with a positive score assigned if > 50% of cells exhibited vacuoles.

Results: A total of 12/30 (40%) of all Hp isolates produced vacuolating cytotoxin. We found 5/16 (31.2%) of DU isolates and 7/14 (50%) of NUD isolates produced vacuolating cytotoxin, which is not a statistically significant difference.

Conclusions: We conclude that the prevalence of vacuolating cytotoxin among Hp isolates is not different between DU and NUD patients, which contradicts previous reports. If there are certain properties which distinguish Hp strains with ulcerogenic potential, our findings would suggest that the presence of vacuolating cytotoxin is not such a property.

THE MOTILITY OF HELICOBACTER PYLORI: COMPARING ISOLATES FROM DUODENAL ULCER AND NON-ULCER DYSPEPSIA PATIENTS. A.J. DeCross, L.J. Barrett, R.L. Guerrant, B.J. Marshall, University of Virginia, Charlottesville, VA.

The motility of Helicobacter pylori (Hp) has been shown to be a key virulence factor in the successful infection of animal models. Our purpose is to compare the prevalence of motile Hp isolates among duodenal ulcer patients (DU's) and non-ulcer dyspepsia patients (NUD's).

Methods: 15 Hp isolates from DU's (non-steroidal medication use excluded) and 11 Hp isolates from NUD's were studied. The two groups were closely matched for patient age, race, and sex. Isolates were initially obtained from antral biopsies which were cultured on horse blood agar plates at 37°C in a 10% CO_2 incubator; they were then frozen in T-soy broth (15% glycerol) at -70°C. Frozen isolates were later recovered by reculturing on horse blood agar plates, and then used within 5 days in the motility assay described by Eaton et al*. Hp was inoculated into 10cc of brucella broth (10% fetal calf serum) to a concentration of 10^6 viable organisms/cc. The broth was cultured in a 50cc Erlenmeyer flask (loose cap) on a Gyrotory shaking platform (150 rpm) in the 37°C 10% CO_2 incubator. After 48 hours, 1 μl of broth was inoculated into 15cc of soft agar (brucella broth with 10% fetal calf serum and .5% agar cooled to 42°C), poured into petri dishes, and kept 4 days in the above incubator. Colony morphology was then examined by phase contrast microscopy, and colony size measured by ocular micrometer. As described by Eaton et al*, amotile colonies (aflagellate) are 250 μm wide and densely granular, while motile colonies (flagellated) vary from 1-5 mm wide and are diffusely spreading.

Results: A total of 15/26 (57.7%) of all Hp isolates contained motile organisms. We found 10/15 (66.7%) of DU isolates and 5/11 (45.5%) of NUD isolates to contain motile organisms (not statistically significant).

Conclusions: The majority of Hp isolates contain motile organisms. However, we could not demonstrate that this property distinguished Hp isolated from DU's in comparison to NUD's. This suggests that motility is not a factor unique to potentially ulcerogenic Hp strains.

(*Eaton K. et al:Infect & Immunity;1989;57:1119)

BETA-HEMOLYSIS: A NEW VIRULENCE FACTOR FOR HELICOBACTER PYLORI. A.J. DeCross, R.L. Guerrant, B.J. Marshall, Dept. of Medicine, University of Virginia, Charlottesville, VA.

Our purpose is to describe the beta-hemolytic activity of Helicobacter pylori (Hp) grown on solid blood agar, and to compare this activity between Hp isolates from duodenal ulcer patients (DU's) vs non-ulcer dyspepsia patients (NUD's).

Methods: We had available Hp isolates from 12 DU's (non-steroidal medication use excluded) and from 12 NUD's matched for age, race, and sex. Isolates were grown on horse blood agar plates (150mm plate, Columbia agar with 7% horse blood) containing GCHI enrichment (REMEL, Lenexa, KS) at 37°C in a 10% CO_2 incubator. Isolates were subcultured twice over 8 days before study. Investigators were blinded to isolate origins. A confluent "carpet" of colony growth was present 2 days after plate inoculation. On days 2 thru 8, the blood agar plates were visually assessed for their degree of hemolysis, which was graded "mild" (faint streaks), "moderate" (full area under "carpet" had defined borders; agar color either smokey or white) and "severe" (could read through the agar in most areas). All cultures were viable at day 8.

Results: No hemolysis was seen until day 3, but all Hp isolates showed some degree of beta-hemolysis by day 7. Although there were an equal number of DU and NUD isolates causing severe hemolysis by day 8, hemolysis progressed more quickly for the DU isolates. Specifically, 9/12 DU isolates achieved moderate hemolysis by day 5 as compared to 3/12 NUD isolates, which is significant at $p < .02$. A sterile control plate showed no hemolysis by day 8.

Conclusions: We conclude that all strains of Hp have some degree of beta-hemolytic activity. Our qualitative pilot study suggests that this activity may be greater in DU compared to NUD isolates. Beta-hemolytic activity is proposed as a new virulence factor for Hp, and deserves further analysis as a contributing factor to the ulcerogenic potential of Hp.