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The Neutrophil Respiratory Burst and Bacterial Digestion in Crohn's Disease

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Abstract

Background—Neutrophils are a key part of the innate immune defence against microbes, using the respiratory burst (RB) to optimise killing and digestion. Previous studies of the neutrophil RB in Crohn's disease (CD) have yielded conflicting results.

Methods—Superoxide production in response to phorbolmyristyl acetate (PMA) was measured in neutrophils from 100 patients with CD compared to 50 healthy controls (HCs) and 50 patients with ulcerative colitis (UC). A further 22 CD and 10 HCs were studied using f-Met-Leu-Phe (fMLP), and digestion of *E. coli* by neutrophils was also evaluated.

Results—The mean $\pm$ SEM PMA-stimulated RB (nmol $O_2/10^6$ cells/min) was 10.86 ± 0.26 in HCs, 9.76 ± 0.23 in CD ($P=0.02$) and 10.04 ± 0.28 in UC ($P=0.09$ vs HC and 0.47 vs CD). No significant effect of age, gender or medication was observed. The RB in three patients with presumed CD was found to be in the range expected in patients with inherited neutrophil disorders. Stimulation with fMLP was calcium dependent and attenuated in patients on 5-ASA. Digestion of *E. coli* by neutrophils was not different in HC vs CD (21.6 vs 20.53%, $P=0.60$).

Conclusion—The significant reduction in neutrophil RB in CD does not appear to result in defective bacterial digestion and is therefore unlikely play a major role in pathogenesis. Three patients in this cohort of patients with presumed idiopathic CD were found to have a profound

defect of the neutrophil RB. A high index of suspicion for such patients is prudent, as their prognosis can be improved by altering or augmenting the conventional treatment regimens employed for CD.

Keywords

Neutrophil; Respiratory burst; Superoxide; Digestion; Crohn's disease

Introduction

Neutrophil phagocytes are essential in the mounting and resolution of the acute inflammatory response—acting as the first line of cellular defense against invading microorganisms [1]. Their antimicrobial function is dependent on the presence and action of digestive enzymes [2, 3], the last being optimized by the respiratory burst (RB). Generated by the membrane-bound NADPH oxidase enzyme complex, the RB results in the production of superoxide (O_2^-).

The function of this enzyme complex is attenuated in chronic granulomatous disease (CGD) [4] and glycogen storage disease type-1b (GSD-1b) [5]. Both are rare inherited disorders associated with intestinal inflammation that is indistinguishable from Crohn's disease (CD), in 30–50% of cases [6], reflecting a pathogenic role for innate immune dysfunction in CD. A number of other monogenic disorders of neutrophil function exist, but are less prevalent than CGD and GSD-1b, do not generally affect NADPH oxidase function, and are less commonly associated with intestinal inflammation (with the notable exception of Chediak–Higashi syndrome, where the association is around 33% [6]).

Given the prevalence of inflammatory bowel disease in patients with inherited neutrophil disorders, it is possible that such patients present to Gastroenterology services without a prior diagnosis of their underlying disorder, thus having their intestinal inflammation classified as idiopathic CD, and then treated accordingly.

Although the pathogenesis of CD still remains to be fully elucidated, there is now increasing evidence that innate immune dysfunction, and more specifically defective acute inflammation, plays a primary role [7, 8]. Impaired neutrophil influx to, and subsequent clearance of, a bacterial stimulus in CD has been attributed to defective secretion of pro-inflammatory cytokines by macrophages [7, 9, 10]. When isolated in vitro, chemotaxis and migration in neutrophils from patients with quiescent CD appears preserved [11, 12].

Previous studies of the neutrophil RB in CD, however, have yielded conflicting results. Using a variety of methods and stimuli, it has been shown either to be increased [13,14], normal [15, 16], or reduced [17, 18]. In addition, the ability of neutrophils in CD to kill *Candida albicans* has been shown to be either reduced [19] or near-normal under physiological conditions [20], with a normal level of superoxide production in response to the same organism [17]. Varying inferences have therefore been made about the pathogenic contribution of these cells.

There has been increasing interest in the impaired handling of bacteria by antigen-presenting cells of the innate immune system in CD [21, 22]. However, the ability of neutrophils in CD to digest bacteria (their primary function [1]) has not been evaluated.

This study was performed on a large cohort of patients with CD to determine whether the neutrophil respiratory burst was attenuated and perhaps related to impaired bacterial digestion.

Methods

Ethical Approval and Patient Selection

Full ethical committee approval was obtained for all experiments in accordance with National Research Ethics Service guidelines (www.nres.org.uk), and written, informed consent was obtained in all cases prior to inclusion (Joint UCL/UCLH committee alpha, ref: 02/0324). Patients attending the IBD clinics at UCLH NHS Foundation Trust with quiescent inflammatory bowel disease (aged 18–65) were selected. Quiescent disease was defined as a Harvey-Bradshaw Index score of 3 or less. Blood tests were only performed at physician discretion, and therefore not all had CRP performed. However, where performed, and in all patients with perianal disease, this test was normal (<5.0 mg/l). Patients with perianal disease met the additional criteria of being on no antibiotics within 2 months of inclusion and having no radiological evidence of perianal sepsis within 2 months (or at time of last scanning—whichever was the nearer), and no draining fistulae or seton in situ at the time of sampling. In general, patients on no medication were preferred, but those on stable doses of mesalazine (5-ASA) (for 3 months prior to inclusion) were also examined. Doses of immunosuppressants or infliximab, where prescribed, had been stable for at least 6 months prior to sampling and patients on infliximab therapy were sampled immediately prior to regular infusion (within 10 min).

Neutrophil Isolation

Briefly, neutrophils were isolated from peripheral venous blood (heparinized 6 U/ml) by gradient centrifugation (Lymphoprep[®], Axis-Shield) and hypotonic lysis to remove red blood cells. Neutrophils were resuspended at required concentrations in phosphate-buffered saline (PBS; Gibco). This method resulted in >95% purity and viability by Trypan Blue exclusion.

RB Assay

Neutrophils (2×10^6) in PBS with 0.54 mg/ml cytochrome-*c* (Sigma) in a final volume of 1 ml were equilibrated to 37°C in a temperature-controlled spectrophotometer (UV-3,000, Shimadzu Corp). The RB was induced by the addition of either 1 µg/ml phorbol myristyl acetate (PMA) or 10 nM f-Met-Leu-Phe (fMLP; both from Sigma). The color change thus produced at 549 nm was used to calculate the amount of superoxide produced (nmol O₂/10⁶ cells/min) using a pre-determined formula [23]. With fMLP, results were compared to a second, paired reaction mixture where neutrophils had been allowed to rest for 15 min in PBS containing 2 mM calcium chloride prior to stimulation.

Preparation of Bacteria for Digestion Assay

A clinical reference strain of *E. coli* (NCTC 10418) was labeled in culture with ^{35}S -methionine, washed to remove unbound label and killed by UV irradiation. Bacteria were then counted, aliquoted, snap-frozen, and stored until use at -70°C . On the day of use, the required amount of bacteria was opsonized with de-salted pooled human IgG (Octagam; Octapharma, UK) resuspended in PBS (Gibco).

Digestion Assay

In triplicate reactions, 2×10^6 neutrophils in PBS with 5 mM glucose were mixed with opsonized, radiolabeled bacteria in a 1:2 ratio, and digestion left to occur in a bovine serum albumin-coated plastic 24-well plate (Corning) at 37°C and 200 rpm. After 60 min, the reaction was stopped by cooling to 4°C . Undigested bacteria and cells were removed by centrifugation at 14,000 rpm and radioactivity counted in the supernatant and pellet. Digestion was calculated from the amount of radioactivity in the supernatant and expressed as a percentage of the total counts in each reaction, as an average of the triplicate reactions. The average variation in each assay around the triplicate mean was 3.51 ± 0.68 units. Expressed as a percentage of the triplicate mean, this was $26.0 \pm 4.91\%$. Diphenylene iodonium (5 μM) (DPI; an inhibitor of NADPH oxidase; Sigma) was used to inhibit digestion.

Statistical Analysis

Data were subjected to the Shapiro–Wilk test for normality and comparisons between two groups were made by Student's *t* test if normally distributed, and two-tailed Mann–Whitney *U* test if not. For multiple group comparisons, one-way ANOVA with Bonferroni post-test comparisons or Kruskal–Wallis ANOVA with Dunn post-hoc test were used, respectively. Graphs and statistical analyses were performed in Prism v4.0 (GraphPad). All results are expressed in tables and figures as mean $\pm$ SEM unless otherwise specified.

Results

PMA-Induced RB

Clinical and demographic details are shown in Table 1. One hundred patients with CD were compared to 50 healthy controls and 50 patients with ulcerative colitis. Mean values $\pm$ SEM for superoxide production (in $\text{nmol O}_2/10^6$ cells/min) after stimulation with PMA (Fig. 1a) were 10.86 ± 0.26 for healthy controls, 9.76 ± 0.23 for patients with CD ($p = 0.02$) and 10.04 ± 0.28 for ulcerative colitis ($p = 0.09$ vs. healthy controls and 0.47 vs. CD). Nine CD and one UC patient released superoxide at levels below the lowest healthy control subject. Of these, three CD patients had superoxide production less than 30% that of the healthy control mean, a level that might be expected from patients with variant CGD [23] (Table 2).

No significant differences were observed between CD patients divided according to disease location (Fig. 1b), although the mean superoxide production in patients with colonic disease (L2) was significantly lower than healthy controls ($p = 0.008$).

Clinical and Demographic Influences on the PMA-Induced RB

There were no significant differences in mean RB between patients on no medication (10.31 ± 0.26), those on 5-ASA (10.49 ± 0.32 , $p = 0.70$), or those on immunosuppressant or biologic therapy (9.79 ± 0.35 , $p = 0.24$). As patients with colonic disease had a significantly reduced RB compared to healthy controls, they were analyzed further with regard to possible influence of anti-inflammatory medications (which might be expected to attenuate the RB). Although significantly different to healthy controls ($p = 0.02$), superoxide production in colonic CD patients on no medication (9.19 ± 0.49) was no different to those on 5-ASA (10.03 ± 0.47 , $p = 0.28$) or those on immunosuppressant or biologic therapy (9.80 ± 0.98 , $p = 0.56$) (Fig. 2a). Mean superoxide production was also unaffected by gender, smoking, or age ($r^2 = 0.00003$, $p = 0.99$ for HC; 0.01, 0.40 for CD) (Fig. 2b–d).

Receptor-Mediated RB

Cells from 11 healthy controls and 22 patients with CD were stimulated with 10 nM fMLP and the rate of superoxide measured (Fig. 3a). Neutrophils from CD patients demonstrated diminished superoxide production compared to healthy controls ($p = 0.03$). No significant differences were observed between CD patients divided according to disease location (Fig. 3b), although the mean superoxide production in patients with ileal disease (L1) was significantly lower than healthy controls ($p = 0.03$). There appeared to be a relationship between an attenuated RB and therapy with 5-ASA (mesalamine; open symbols Fig. 3b) and previous studies have demonstrated a calcium-dependent attenuation of the fMLP-induced RB in vitro with this drug [28, 29]. In keeping with this observation, pre-incubation of neutrophils with calcium corrected the attenuated RB observed in patients on mesalamine (Fig. 3c).

Bacterial Digestion

Diminished neutrophil NADPH oxidase activity in patients with disorders such as CGD and GSD-1D has been shown to result in defective bacterial digestion. No significant difference in bacterial digestion was observed between healthy controls ($n = 15$) and patients with intestinal CD ($n = 16$; $p = 0.18$, Fig. 4). Patients with perianal disease ($n = 15$) were analyzed as a separate group due to the strong link between inherited neutrophil disorders and perianal sepsis [6] (although the RB was not found to be abnormal in such patients). In all cases, digestion was partially inhibited by the addition of 5 μ M diphenylene iodonium (DPI) a known inhibitor of the NADPH oxidase. Neutrophils from the three patients with an abnormal RB were found to have markedly diminished digestive capacity compared to healthy controls and patients with CD.

Patients were subdivided according to phenotype and medication to determine whether the differences observed in RB affected bacterial digestion. Neither patients with colonic disease nor those on 5-ASA demonstrated a significant attenuation of digestive capability (Fig. 4b, c).

Discussion

A profound defect of superoxide production in a number of rare inherited disorders is strongly associated with intestinal inflammation that is indistinguishable from CD [6] and it has been well documented that the loss of NADPH oxidase activity results in a reduced capacity of neutrophils to digest phagocytosed microbes [4, 24]. We have recently shown that CD patients have delayed bacterial clearance in vivo as the result of diminished neutrophil recruitment [7]. While this could be linked to attenuated pro-inflammatory cytokine secretion by macrophages, given previous conflicting descriptions of neutrophil dysfunction in CD, it was important to determine whether a relevant intrinsic neutrophil defect could be demonstrated.

Our findings demonstrate that a small but significant reduction in superoxide production in CD patients (particularly those with L2 disease) does not have an effect on the digestive capability of the neutrophil. An interesting finding arising from this study was the identification of three patients with superoxide level in the region usually associated with variant CGD. Subsequently, one patient has been shown to have GSD-1b and another a congenital neutropenia (CN) syndrome arising from a mutation in *G6PC3*, both of which result in attenuated NADPH oxidase activity and superoxide production. The third patient had neither CGD nor the above two conditions and her defect remains uncharacterized.

It is interesting that polymorphisms in *NCF4*, encoding the NADPH oxidase component p40^{phox}, have been associated with CD, although only with ileal (L1) disease and not in all Caucasian populations [25]. The small, but statistically significant reduction, in mean superoxide production by CD neutrophils may be the result of a combination of influences across the group as a whole—including age, medication, disease location, or underlying genetic influences. When analyzed alone, however, the influence of each of these factors (although *NCF4* polymorphisms were not investigated in this study) is statistically non-significant.

The use of fMLP as a stimulus produced differing results. Formyl peptide receptor (FPR) signaling induced by fMLP results in activation of the NADPH oxidase complex via a calcium-dependent pathway involving phospholipase-C [26]. The correction in RB observed when neutrophils were pre-incubated with calcium implies that patients with CD have reduced intracellular calcium stores or have deficient mobilization of calcium in response to fMLP. These results would be in keeping with the finding of reduced intracellular calcium levels in patients with quiescent CD on maintenance therapy with 5-ASA [27]. The capacity of 5-ASA and related compounds to inhibit the fMLP-induced neutrophil RB in vitro [28, 29] also appear to be reflected in our results. There are many neutrophil functions that are associated with or dependent on the release of intracellular calcium [30, 31]. The “antibacterial” consequences of the diminished fMLP-induced RB are unclear since bacterial digestion appeared preserved. It is unlikely, therefore, that the variable response to fMLP indicates a primary or pathogenically significant defect.

Defective bacterial handling has been linked to the development of CD [22, 32], particularly in epithelial cell lines [33] or macrophages [34] from CD patients expressing *ATG16L1*

polymorphisms. While these polymorphisms were not studied as part of this investigation, our results demonstrate normal digestion in neutrophils from patients with CD, suggesting that the CD-associated polymorphism is more relevant to bacterial processing and antigen presentation in the above cells [35] rather than primary antibacterial activity in neutrophils.

Three patients in this large adult cohort were found to have an RB in the range expected in CGD. Further analysis of these patients has been submitted elsewhere. Although the proportion of adult patients with an undiagnosed primary neutrophil disorder is likely to be small, the advantage of identifying such patients in a cohort with presumed idiopathic CD is considerable. It may be advantageous to “screen” for neutrophil dysfunction in this cohort—perhaps concentrating on patients with an early onset of disease, a history of recurrent bacterial infections, “unusual” (cf. disease location) or therapy-recalcitrant inflammatory bowel disease. Oral involvement was a feature of all three patients with an abnormal RB and no patients with CD and a “normal” RB had oral involvement at the time of sampling. However, our method of classification may not reliably detect patients with a previous history of oral involvement at some stage in their disease course. A similar “screening” study of the RB in patients with oral involvement is planned.

The impaired clearance of bacteria seen in patients with CD has been attributed to attenuated neutrophil influx [7] and can now be thought of in terms of reduced neutrophil number rather than function. Innate immunodeficiency in CD does not appear to involve an intrinsic defect of neutrophil antimicrobial function.

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Reference

1. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol.* 2005; 23:197–223. [PubMed: 15771570]
2. Reeves EP, Lu H, Jacobs HL, et al. Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. *Nature.* 2002; 416:291–297. [PubMed: 11907569]
3. Belaouaj A, McCarthy R, Baumann M, et al. Mice lacking neutrophil elastase reveal impaired host defense against Gram-negative bacterial sepsis. *Nat Med.* 1998; 4:615–618. [PubMed: 9585238]
4. Segal AW. The NADPH oxidase and chronic granulomatous disease. *Mol Med Today.* 1996; 2:129–135. [PubMed: 8796870]
5. Annabi B, Hiraiwa H, Mansfield BC, et al. The gene for glycogen-storage disease type 1b maps to chromosome 11q23. *Am J Hum Genet.* 1998; 62:400–405. [PubMed: 9463334]
6. Rahman FZ, Marks DJ, Hayee BH, et al. Phagocyte dysfunction and inflammatory bowel disease. *Inflamm Bowel Dis.* 2008; 14:1443–1452. [PubMed: 18421761]
7. Smith AM, Rahman FZ, Hayee B, et al. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn’s disease. *J Exp Med.* 2009; 206:1883–1897. [PubMed: 19652016]
8. Casanova JL, Abel L. Revisiting Crohn’s disease as a primary immunodeficiency of macrophages. *J Exp Med.* 2009; 206:1839–1843. [PubMed: 19687225]

9. Marks DJ, Harbord MW, MacAllister R, et al. Defective acute inflammation in Crohn's disease: A clinical investigation. *Lancet*. 2006; 367:668–678. [PubMed: 16503465]
10. Zhou L, Braat H, Faber KN, et al. Monocytes and their pathophysiological role in Crohn's disease. *Cell Mol Life Sci*. 2009; 66:192–202. [PubMed: 18791847]
11. Richens ER, Williams MJ, Gough KR, et al. Leucocyte migration studies in Crohn's disease using Crohn's colon homogenate and mitochondrial and microsomal fractions. *Gut*. 1974; 15:19–23. [PubMed: 4820628]
12. Rhodes JM, Jewell DP. Motility of neutrophils and monocytes in Crohn's disease and ulcerative colitis. *Gut*. 1983; 24:73–77. [PubMed: 6848434]
13. Biagioni C, Favilli F, Catarzi S, et al. Redox state and O₂^{*}-production in neutrophils of Crohn's disease patients. *Exp Biol Med* (Maywood). 2006; 231:186–195. [PubMed: 16446495]
14. Faden H, Rossi TM. Chemiluminescent response of neutrophils from patients with inflammatory bowel disease. *Dig Dis Sci*. 1985; 30:139–142. [PubMed: 3967561]
15. Verspaget HW, Mieremet-Ooms MA, Weterman IT, et al. Partial defect of neutrophil oxidative metabolism in Crohn's disease. *Gut*. 1984; 25:849–853. [PubMed: 6086464]
16. Williams JG, Hughes LE, Hallett MB. Toxic oxygen metabolite production by circulating phagocytic cells in inflammatory bowel disease. *Gut*. 1990; 31:187–193. [PubMed: 2311976]
17. Curran FT, Allan RN, Keighley MR. Superoxide production by Crohn's disease neutrophils. *Gut*. 1991; 32:399–402. [PubMed: 1851125]
18. Gionchetti P, Campieri M, Guarnieri C, et al. Respiratory burst of circulating polymorphonuclear leukocytes and plasma elastase levels in patients with inflammatory bowel disease in remission. *Dig Dis Sci*. 1994; 39:550–554. [PubMed: 8131691]
19. Caradonna L, Amati L, Lella P, et al. Phagocytosis, killing, lymphocyte-mediated antibacterial activity, serum autoantibodies, and plasma endotoxins in inflammatory bowel disease. *Am J Gastroenterol*. 2000; 95:1495–1502. [PubMed: 10894586]
20. Curran FT, Youngs DJ, Allan RN, et al. Candidacidal activity of Crohn's disease neutrophils. *Gut*. 1991; 32:55–60. [PubMed: 1991639]
21. Xavier RJ, Huett A, Rioux JD. Autophagy as an important process in gut homeostasis and Crohn's disease pathogenesis. *Gut*. 2008; 57:717–720. [PubMed: 18272545]
22. Klionsky DJ. Crohn's disease, autophagy, and the Paneth cell. *N Engl J Med*. 2009; 360:1785–1786. [PubMed: 19369659]
23. Woodman RC, Newburger PE, Anklesaria P, et al. A new X-linked variant of chronic granulomatous disease characterized by the existence of a normal clone of respiratory burst-competent phagocytic cells. *Blood*. 1995; 85:231–241. [PubMed: 7803797]
24. Quie PG, White JG, Holmes B, et al. In vitro bactericidal capacity of human polymorphonuclear leukocytes: Diminished activity in chronic granulomatous disease of childhood. *J Clin Invest*. 1967; 46:668–679. [PubMed: 6021213]
25. Roberts RL, Hollis-Moffatt JE, Gearry RB, et al. Confirmation of association of IRGM and NCF4 with ileal Crohn's disease in a population-based cohort. *Genes Immun*. 2008; 9:561–565. [PubMed: 18580884]
26. Hurst NP. Molecular basis of activation and regulation of the phagocyte respiratory burst. *Ann Rheum Dis*. 1987; 46:265–272. [PubMed: 3036025]
27. Schmidt C, Kosche E, Baumeister B, et al. Arachidonic acid metabolism and intracellular calcium concentration in inflammatory bowel disease. *Eur J Gastroenterol Hepatol*. 1995; 7:865–869. [PubMed: 8574719]
28. Nielsen OH, Bouchelouche PN, Berild D, et al. Effect of 5-aminosalicylic acid and analogous substances on superoxide generation and intracellular free calcium in human neutrophilic granulocytes. *Scand J Gastroenterol*. 1993; 28:527–532. [PubMed: 8100641]
29. Neal TM, Winterbourn CC, Vissers MC. Inhibition of neutrophil degranulation and superoxide production by sulfasalazine. Comparison with 5-aminosalicylic acid, sulfapyridine and olsalazine. *Biochem Pharmacol*. 1987; 36:2765–2768. [PubMed: 2888463]
30. Tintinger G, Steel HC, Anderson R. Taming the neutrophil: Calcium clearance and influx mechanisms as novel targets for pharmacological control. *Clin Exp Immunol*. 2005; 141:191–200. [PubMed: 15996182]

31. Brechard S, Melchior C, Plancon S, et al. Store-operated Ca^{2+} channels formed by TRPC1, TRPC6 and Orai1 and non-store-operated channels formed by TRPC3 are involved in the regulation of NADPH oxidase in HL-60 granulocytes. *Cell Calcium*. 2008; 44:492–506. [PubMed: 18436303]
32. Glasser AL, Darfeuille-Michaud A. Abnormalities in the handling of intracellular bacteria in Crohn's disease: A link between infectious etiology and host genetic susceptibility. *Arch Immunol Ther Exp (Warsz)*. 2008; 56:237–244. [PubMed: 18726145]
33. Kuballa P, Huett A, Rioux JD, et al. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease-associated ATG16L1 variant. *PLoS One*. 2008; 3:e3391. [PubMed: 18852889]
34. Hudspith B, Rayment N, Prescott N, et al. In vitro responses of macrophages to *E. coli* challenge in Crohn's disease. *Gastroenterology*. 2009; 136:A250.
35. Fujita N, Saitoh T, Kageyama S, et al. Differential involvement of Atg16L1 in Crohn disease and canonical autophagy: Analysis of the organization of the Atg16L1 complex in fibroblasts. *J Biol Chem*. 2009; 284:32602–32609. [PubMed: 19783656]

Fig. 1.

The PMA-stimulated neutrophil RB. (a) The RB was studied in response to PMA in patients with CD and UC compared to HCs (*dotted line* denotes 30% of healthy control mean; *Labeled points* correspond to patients 1–3 in Table 2). (b) Patients with CD divided into groups according to disease location (Montreal classification). * $p < 0.05$, ** $p < 0.01$. All other comparisons were non-significant

Fig. 2. Demographics and the PMA-stimulated RB. The effect of medication (a) gender (b), and smoking status (c) on the PMA-stimulated RB is shown in patients with CD and HCs. (d) Linear correlation of age and PMA-stimulated RB with patients 1–3 removed from correlation (HC: dotted line; $r^2 = 0.000004$, $p = 0.99$; CD: solid line; $r^2 = 0.03$, $p = 0.18$)

Fig. 3.

The fMLP-stimulated RB. **(a)** The RB was studied in response to fMLP in patients with CD compared to HC. **(b)** Patients with CD divided by disease location. Those on mesalamine are shown as *open symbols* while those on no medication are denoted by *filled symbols*. **(c)** Paired reactions were carried out with or without pre-incubation with calcium (Ca²⁺). * $p < 0.05$, ** $p < 0.01$ (paired t test). All other comparisons were non-significant

Fig. 4.

Digestion of *E. coli* by neutrophils. (a) The release of radioactivity into the supernatant of a reaction with neutrophils and ^{35}S -labeled *E. coli* was measured in HC, CD, and patients with perianal involvement (P). Patients 1–3 (Table 2) were analyzed as a separate group. Patients with CD were subdivided according to (b) disease location and (c) patients on treatment with 5-ASA were compared to those on no treatment ** $p < 0.01$. All other comparisons were non-significant

Table 1

Characteristics of study subjects

	<u>With PMA</u>			<u>With fMLP</u>		<u>For bacterial digestion</u>	
	HC	CD	UC	HC	CD	HC	CD
<i>n</i> =	50	100	50	11	22	15	31
Gender (M:F)	27:23	40:60	23:27	4:7	6:16	9:6	14:17
Mean age $\pm$ SD (years)	41.7 $\pm$ 7.2	41.5 $\pm$ 15.1	45.9 $\pm$ 15.2	27.2 $\pm$ 4.6	39.6 $\pm$ 14.8	36.8 $\pm$ 8.2	42.5 $\pm$ 4.9
Current smokers	9	20	12	1	0	0	0
No medication	–	27	14	–	7	–	10
Mesalazine only	–	49	25	–	14	–	11
IS/Anti-TNF	–	32	11	–	1	–	10
<i>Disease location</i>							
L1	–	24	–	–	9	–	5
L2	–	40	–	–	10	–	6
L3	–	29	–	–	3	–	5
Perianal	–	15	–	–	–	–	15

IS immunosuppressant (azathioprine or methotrexate)

Table 2

Three patients with an abnormal respiratory burst

	1	2	3
Gender	M	F	F
Age at diagnosis (years)	9.4	9.1	10.0
Current age (years)	27	23	21
Disease location	Oral; L2	L2	Oral; L3
Treatment ^a	MTX	AZA	5-ASA
O ₂ ⁻ production ^b	0.1	2.7	3.3
Final diagnosis	<i>G6PC3</i> -CN	GSD-1b	?

CN congenital neutropenia,

^a patient 3 previously treated with azathioprine,^b in nmolO₂/10⁶ cells/min, on three separate occasions