Pediatric inflammatory bowel disease: Diagnostics, treatment and psychosocial consequences
Hummel, T.Z.

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CHAPTER 1

Exogenous pigment in Peyers’ patches of children suspected for inflammatory bowel disease

Thalia Z. Hummel
Angelika Kindermann
Pieter C.F. Stokkers
Marc A. Benninga
Fiebo J.W. ten Kate

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ABSTRACT

Objectives: The base of human Peyer’s patches of the terminal ileum has been noted to contain black granular pigment deposits, composed of titanium dioxide and aluminosilicate, which are food additives typically present in a Western diet and pharmaceuticals. In this study we investigated the distribution of exogenous pigment throughout the gastrointestinal tract of children suspected for inflammatory bowel disease (IBD), the correlation between their age and the presence and amount of pigment in Peyer’s patches and its relation with pediatric IBD.

Methods: Biopsies (upper and lower gastrointestinal tract) from children suspected for IBD who underwent endoscopy, were reassessed by a blinded, expert pathologist. The amount of pigment in biopsies was scored using a semiquantitative scale (range 0 to +++).

Results: A total of 151 children were included: 62 Crohn’s disease (CD), 26 Ulcerative Colitis (UC), and 63 non-IBD. In 63 children (42%) deposits of black pigment were found, only in biopsies from the terminal ileum, located in Peyer’s patches. A significant correlation was found between increasing age and the amount of pigment (p=0.004). Pigment deposits were found significantly less in CD patients compared to UC and non-IBD patients (26% versus 62% and 49%, p=0.002).

Conclusions: These results provide support for the hypothesis that the amount of pigment, only present in Peyer’s patches in the terminal ileum, becomes denser with increasing age. Absence of pigment in Peyer’s patches in a higher number of CD patients suggests that microparticles might have become involved in the inflammatory process, possibly due to disrupted autophagy.
INTRODUCTION

The incidence of childhood-onset Crohn’s disease (CD) and ulcerative colitis (UC) has dramatically increased in Western countries in the last decades (1). The etiology of inflammatory bowel disease (IBD) is unknown, but evidence suggests that it results from a combination of genetic predisposition and environmental factors (2). The latter is clearly important since IBD is almost exclusively a disease of developed countries. Recent data have shown a rapidly increasing incidence in Asia, which seems to occur in parallel with the rapid socioeconomic development taking place (3,4). A change in diet to a more Westernized diet may underlie this epidemiological change in the Asian population (4). Indeed, diet has been the subject of much discussion and speculation. However, most studies dealing with this subject have only provided indirect evidence of a possible cause-and-effect relationship between specific dietary factors and IBD (2). Upon light microscopy, Shepperd et al. have found that the base of human Peyer’s patches of the terminal ileum contains black granular pigment deposits (5). Further analysis has demonstrated that these pigment deposits consist of titanium dioxide and aluminosilicate (5,6). There are two hypotheses concerning the route these microparticles might reach the Peyer’s patches. The first hypothesis assumes that these metals and minerals in the bowel are derived from ingested materials, such as food additives, pharmaceuticals and toothpaste (7,8). The second hypothesis assumes that materials from the atmospheric dust are inhaled and that macrophages in the lung bearing these materials are expectorated and swallowed (8). Following digestion these materials are re-ingested by macrophages in the bowel. A variation of this hypothesis is that the macrophages in the lung are deposited in the bowel via the bloodstream or lymphatic vessels (8). Based on the anatomical distribution of these microparticles in the Peyer’s patches of the terminal ileum it has been suggested that these particles may play a role in the etiology of CD (9). Until now little is known about the presence of exogenous pigment in the gastrointestinal tract of children. Pigment deposits only have been reported in a few children, all older than 6 years (5). It has been suggested that very young children, while being exposed to these exogenous materials, may not have accumulated enough pigment in the Peyer’s patches to be detected by light microscopy (5). Therefore, the aims of this study were: 1) to map the distribution of exogenous pigment throughout the gastrointestinal tract of children suspected for IBD, 2) to assess the correlation between age and the presence and amount of exogenous pigment in the Peyer’s patches of the terminal ileum and 3) to determine its relation with pediatric IBD.
MATERIALS AND METHODS

All children suspected for IBD visiting our department of pediatric gastroenterology between January 2003 and December 2008 were selected for this study. As part of the clinical work-up, all patients underwent both, ileo-colonoscopy and upper gastrointestinal tract endoscopy. Only children in whom the terminal ileum was intubated were included. All patients had at least one, mostly 2 or more biopsy specimens taken from each part of the colon (cecum, ascending colon, transverse colon, descending colon and rectum), terminal ileum, duodenum, stomach (antrum and corpus) and esophagus. Biopsies were taken from macroscopically normal mucosa and from inflamed areas. The tissue was formalin fixed, paraffin embedded and routinely processed and stained with hematoxylin and eosin. Each biopsy specimen was cut in two or more levels to increase the chance to detect pigment. Biopsies from all sites were reassessed, using light microscopy, by an expert pathologist, who was blinded to the clinical condition. The amount of pigment was scored using a semiquantitative scale, range 0 to (+++). In the (+) group only some spots of pigment were seen. The biopsies which were full of pigment, were scored as (+++). All biopsies that contained less pigment than (+++) and more than (+) were grouped into (++). Patients were divided into three groups based on their diagnosis: CD, UC and no inflammatory bowel disease (non-IBD). The diagnosis was made based on the reference standard procedure, which consisted of endoscopic findings and histopathological interpretation, imaging studies and on clinical follow-up data and/or repeated endoscopy. Presenting symptoms, duration of symptoms and clinical follow-up data were extracted from medical charts. A Statement of No Objection was released by our Institutional Review Board.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 20 was used for all analyses. Fisher’s exact tests were used comparing categorical variables between groups and non-parametric tests (Kruskal-Wallis) comparing continuous variables, such as age and duration of disease. Spearman’s correlation coefficient was used to assess the relation between the amount of pigment and age at endoscopy. The criterion for statistical significance was defined as a P-value of <0.05.

RESULTS

Patient characteristics

A total of 172 children suspected of IBD were selected. The terminal ileum was successfully intubated in 151/172. Of these 151 children 54% were male, with a mean age of 12.2 yr (age range: 1.6-18.1 yr). Based on our reference standard diagnostic procedures
62 children fulfilled the criteria for CD, 26 children for UC and 63 children did not have IBD. No differences were found between the 3 main groups with regard to age at endoscopy, gender and duration of symptoms. The follow-up period was significant different between groups (p<0.0001). These data are shown in table 1. None of the non-IBD patients developed IBD or other gastro-intestinal disease during the follow-up period.

### Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>CD N=62</th>
<th>UC N=26</th>
<th>Non-IBD N=63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>22 (35)</td>
<td>15 (58)</td>
<td>33 (52)</td>
</tr>
<tr>
<td>Male, no. (%)</td>
<td>40 (65)</td>
<td>11 (42)</td>
<td>30 (48)</td>
</tr>
<tr>
<td>Age at endoscopy (years), median (Q1,Q3)</td>
<td>12.8 (10.6-15.1)</td>
<td>13.9 (11.3-15.5)</td>
<td>11.8 (8.9-15.0)</td>
</tr>
<tr>
<td>Duration of symptoms (months), median (Q1,Q3)</td>
<td>4 (2-12)</td>
<td>4 (2-7)</td>
<td>6 (3-12)</td>
</tr>
<tr>
<td>Follow-up period (months), median (Q1,Q3)</td>
<td>56 (37-70.2)*</td>
<td>35 (21.5-53.5)*</td>
<td>9 (3-40)*</td>
</tr>
</tbody>
</table>

Q1,Q3= first and third quartile, *significant difference between groups, P<0.05.

**Distribution of pigment**

In 63 children (42%) aggregates of fine black pigment were found in biopsies from the terminal ileum (figure 1a&1b.). The pigment was located in the Peyer’s patches, just within the lymphoid follicle, outside the germinal centre. Pigment cells were not observed in lymphoid follicles in the duodenum, colon or elsewhere in the gut.

**Figure 1a. & 1b.** Hematoxylin and eosin stained slide of distal ileum with black granular pigment in macrophages, located in the Peyer’s patch
Pigment in relation to age
The age of children with pigment in the Peyer’s patches ranged from 3.7 to 18.1 years (mean age 12.1 yr, SD 3.8). There was a significant correlation between age at endoscopy and the amount of pigment (p=0.004, figure 2.). The amount of pigment became denser with increasing age.

Figure 2. Amount of pigment in peyer’s patches in relation to age

Pigment in relation to diagnosis
Pigment was found in a significant lower number of CD patients compared to UC patients and non-IBD patients (26% versus respectively 62% and 49%, p=0.002, figure 3.). Terminal ileitis was found in 11 of 16 CD patients (69%) with pigment in the terminal ileum biopsies and in 36 of 46 CD patients (78%) with no pigment in the terminal ileum (p=0.5). Lymphoid tissue was found in terminal ileum biopsies of 50% of CD patients, 70% of UC patients and 72% of non-IBD patients (p=0.4). In this group of children with lymphoid tissue present in the terminal ileum biopsies, pigment was still found significantly less in CD patients compared to children with UC and non-IBD (p=0.02).
DISCUSSION

This study shows that pigment deposits were present in 42% of all children undergoing endoscopy because of complaints suspected of IBD, only located in the Peyer’s patches in the terminal ileum biopsies. Furthermore, a significant correlation was found between the amount of pigment in the Peyer’s patches and age, with the amount of pigment becoming denser with increasing age. Surprisingly, these pigment deposits were found significantly less in CD patients compared to children with UC and non-IBD.

Shepherd et al. were the first describing their findings of “pigment cells” at the base of Peyer’s patches in 1987 in samples of human small intestine (5). Since then these pigment cells have been described several times, but never in a cohort of children (6,8,9). In accordance with the report of Shepherd et al., in this study pigment deposits were only observed in Peyer’s patches, not in gut associated lymphoid tissue in other parts of the gastrointestinal tract (5). Powell et al. have shown pigment also in lymphoid aggregates in the colon/ileocecal region in a few adult patients (6). Pigment deposits have also been seen
in the endothelial cells of blood vessels (n=2) (8), around delated lymphatics in the submucosa of the ileum (n=2), in mesenteric lymph nodes, and in one patient with ileal Crohn’s disease in transmural inflammatory aggregates (5). However, these studies all examined intestinal resection preparates, which can explain the difference with this studies were only biopsies were available.

Pigment cells are either mature or maturing macrophages containing lysosomes that are full of dense, small microparticles that do not allow transmission of light and thus appear black upon regular light microscopy (6,9). These microparticles appear to be principally aluminosilicates, titanium dioxide and a small proportion of non-aluminium containing silicates (6). Titanium dioxide is a common whitening and brightening agent used in the food- and pharmaceutical industry. Aluminosilicates are widely used in the food industry as anti-caking and thickening agent, as pharmaceutical excipient and in toothpaste (6,10). Intakes of aluminosilicates are greater than for titanium dioxide, respectively 35 and 2,5 mg per person per day in the UK (6). Microparticles can enter the body by crossing the intestinal epithelium via endocytic M-cells overlying intestinal lymphoid aggregates before reaching macrophages. M-cells are specialized, differentiated epithelial cells, which function as an entry and have an exceptional capacity in the uptake of exogenous particles (11,12). The exogenous particles are resistant to enzymatic or chemical degradation and are accumulated in the pigment cells, which are of low metabolic and immunological activity (9). Our finding that the density of pigment deposits increases with age confirms that pigment cells are inert storages of exogenous particles, taken up by the gut, which become saturated in the course of years. Shepherd et al. have shown that pigment in Peyer’s patches is present in adults and children over the age of 6 years (5). However, we have shown in our study that pigment in Peyer’s patches can be found in even younger children. In our cohort six children with pigment deposits in Peyer’s patches were younger than 6 years of age (2 MC, 1 UC, 3 non-IBD). Apparently, in these young children exogenous microparticles have accumulated enough to be detected by light microscopy.

Based upon the site of uptake of these particles in the Peyer’s patches in the terminal ileum, the “classical” site of inflammation in CD, they might play a role in the pathogenesis of CD. It is well-known that similar particles can cause granulomatous diseases in other organs (13,14). Our study shows that pigment in Peyer’s patches was found in a significant lower number of CD patients compared to UC patients and non-IBD patients. We hypothesize that in CD patients the pigment cells have turned into an immunological active state and have become involved in the inflammatory process. Former studies have found that exogenous microparticles can act as adjuvants in the presence of lipopolysaccharide (LPS), significantly enhancing IL-1β (titanium dioxide, in the presence of additional calcium cations) (15,16), IL-10, IL-8 and TNFα responses in vitro and can impair macrophage phagocytic capacity (titanium dioxide and aluminosilicate) (17).
Based on genome wide association studies it has become clear that a defective autophagy plays an important role in the pathogenesis of Crohn’s disease. NOD2 variants have been implemented in MDP-induced autophagy and bacterial targeting towards lysosomes. Dendritic cells that express the disease associated variants have been shown to be defective in both these functions (18). The disease associated variant in the ATG16L1 gene is involved in the same pathway and has been implicated in antimicrobial peptide release and negative regulation of proinflammatory cytokine production (19). Thus, the role of autophagy in the immune response is highly pleiotropic as it is involved in both innate and adaptive immune response and dependent on cell type and interactions with specific microbial factors (20).

Our observation that pigment is found significantly less in the lysosomes of Crohn’s disease patient raises the question whether this observation is associated with autophagy related disease variants and prompts future research in this direction.

An alternative explanation for our observation could be that the biopsies just represent a fraction of the total terminal ileum, which may lead to sampling bias in CD patients if biopsies have been taken from chiefly inflamed mucosa. The terminal ileum biopsies of CD patients contained significant less lymphoid tissue compared to the others. However, analyzing only the group of children with lymphoid tissue present in the terminal ileum biopsies, pigment was still found significantly less in CD patients compared to UC and non-IBD patients, which makes sampling bias less likely.

In summary, in children pigment cells are only present in Peyer’s patches in the terminal ileum, not in gut associated lymphoid tissue in other parts of the gastrointestinal tract. Pigment in Peyer’s patches can be found in children over the age of 3.7 years and the density increases with age. Furthermore, pigment in Peyer’s patches was found in a significant lower number of CD patients compared to children with UC and non-IBD. Knowing that the etiology of IBD is still unclear, the absence of pigment in Peyer’s patches in CD patients strengthens the evidence that microparticles might play a role in the inflammatory process of CD, which might be the result of a defective autophagy pathway. However, this hypotheses remains to be established.
CHAPTER 1

REFERENCES


