INFLAMMATORY FIBROID POLYP: An immunohistochemical study

Gilda da Cunha SANTOS¹, Venâncio A.F. ALVES³, Alda WAKAMATSU³ and Sérgio ZUCOLOTO²

ABSTRACT - Background - Inflammatory fibroid polyp is a localized lesion, which arises in the submucosa of the gastrointestinal tract, most often in the stomach. Although it is generally believed to represent a reactive, nonneoplastic condition, its histogenesis remains controversial. Aim - To study inflammatory fibroid polyp by immunohistochemistry in an attempt to further clarify their histogenesis. Material and Methods - Nine cases were studied by immunohistochemistry using a panel of antibodies against smooth-muscle actin, vimentin, S-100 protein, factor VIII - R.Ag and macrophage (HAM-56). Results - There was a strong diffuse positive staining pattern in the spindle cells with vimentin antibody. A patchy staining for smooth-muscle actin was observed in these cells. Immunophenotyping revealed a heterogeneous reaction with HAM-56. In edematous areas, HAM-56-positive cells show voluminous cytoplasm and reniform nuclei. In cell-rich areas, the HAM-56-positive cells had fusiform cytoplasm. Stains for S-100 and factor VIII R.Ag were negative in the proliferating elements. Conclusions - The present immunohistochemical study refutes the suggested neural or vascular nature of the lesion. The strong positivity for vimentin in all cases suggests a major component of spindle cells best recognizable as fibroblasts. These results would favor the existence of a span of morphological and immunohistochemical patterns possibly indicating evolutionary phases of an inflammatory reaction.


INTRODUCTION

Inflammatory fibroid polyp (IFP) is a rare sessile or pedunculated polyoid lesion of the gastrointestinal tract. Histologically it is composed of mesenchymal elements, loosely arranged collagen fibers, arterioles, blood and lymph capillaries and infiltration with eosinophilic leukocytes and lymphocytes, the latter being occasionally accumulated in rudimentary lymph follicles. Its histogenesis remains controversial. Immunohistochemical studies have largely refuted the previously suggested neural or vascular nature of the lesion by the negative staining for S-100 and factor VIII - related antigen in the proliferating cells. Other markers such as desmin, myoglobin and actin have been frequently negative, strongly suggesting that these cells are not smooth muscle cells. The positivity for lysozyme, alpha 1-anti-trypsin, CD68 and anti-Factor XIIa demonstrated in some cases has supported a histiocytic or a fibrohistiocytic nature of the lesion. The results of some ultrastructural and immunohistochemical studies, however, did not detect the latter aspects and favoured the idea that the principal cells are fibroblasts or primitive mesenchymal cells. Others have demonstrated the presence of fibroblastic, myofibroblastic and histiocytic lines of differentiation in the main cellular component. Recently, it was suggested that inflammatory fibroid polyps with concentric stromal proliferations may originate from a subpopulation of dendritic interstitial cells.

In this study, nine IFPs were characterized immunohistochemically using a panel of antibodies in an attempt to solve the controversies surrounding their histogenesis.

MATERIALS AND METHODS

Nine cases of IFP of the gastrointestinal tract were analyzed. Three cases were collected from the files of the Department of Pathology of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo and another six cases from private laboratories of Ribeirão Preto, SP, and Aracaju, SE. Clinical information was obtained from surgical pathology reports. All tissues had been fixed in formalin and embedded in paraffin. In addition to hematoxylin-and-eosin-stained slides, sections were prepared from paraffin blocks for immunohistochemical stains.

Immunostaining was performed on deparaffinized sections using an immunoperoxidase method with the avidin-biotin amplification system as previously described. A panel of immunohistochemical reactions was performed on a selected block in each case. The
specimens were deparaffinized in xylene and rehydrated. Endogenous peroxidase was blocked with 6% H₂O₂ in methanol for 5 minutes. The primary antibodies, sources and dilutions used are listed in Table 1. All primary antibodies were incubated overnight at 4°C and were sequentially followed by biotinylated rabbit-antimouse or goat-antirabbit IgG antiserum for 30 minutes at 37°C and then by a complex of avidin and biotinylated horseradish peroxidase (Vector Labs, USA) for 30 min at 37°C. Careful rinses with several changes of phosphate-buffered saline were performed between each stage of the procedure. The reaction was developed with a solution of 60 mg% diaminobenzidine in Tris-HCl buffer, pH 7.4, with 0.03% H₂O₂ for 5 minutes in the dark.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Clone</th>
<th>Enzymatic predigeneration agent</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>-</td>
<td>1:100</td>
<td>DAKO</td>
</tr>
<tr>
<td>Smooth-muscle actin</td>
<td>A4</td>
<td>-</td>
<td>1:1600</td>
<td>Sigma</td>
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<tr>
<td>S-100 protein</td>
<td>polyclonal</td>
<td>-</td>
<td>1:6000</td>
<td>DAKO</td>
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<tr>
<td>Macrophage</td>
<td>HAM-56</td>
<td>Trypsin</td>
<td>1:2000</td>
<td>Enzo</td>
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<tr>
<td>Factor VIII-R-Ag</td>
<td>polyclonal</td>
<td>Trypsin</td>
<td>1:2000</td>
<td>DAKO</td>
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**RESULTS**

Clinical findings

The nine patients (Table 2) were five males and four females ranging in age from 35 to 58 years.

Signs and symptoms were abdominal pain (three cases), gastrointestinal intussusceptions (three cases), blood in stools (one case), pyrosis (one case) and vomiting (three cases). In one case, clinical information was not available.

The anatomical sites of the tumors were stomach-antrum (three cases), small bowel (three cases), cecum (one case) and colon (two cases).

**Pathologic findings**

**Gross features** – The polyps ranged from 1.5 to 4.0 cm in greatest diameter. The polypoid lesions usually had a broad base and a tan or gray cut surface. The overlying mucosa usually showed foci of erosion.

**Microscopic features** – Histologically there was a spectrum of features varying from case to case. The majority of the lesions consisted of mixed proliferation of spindle cells, loosely arranged in sweeping fascicles, or whorled structures with poorly defined margins, projecting into the lumen of the bowel. In cases with intact mucosa the margin of the tumor was limited by muscularis mucosa, which was focally fragmented and infiltrated (2 and 7). The lesion usually filled the submucosa interrupting and splitting the muscle coats. In one case (8), the muscularis propria was partially replaced and even the subserosa was infiltrated. Prominent vascularity was noted in all polyps. Some vessels had the appearance and caliber of arterioles and venules.

The lesions were predominantly composed of mononuclear spindle-shaped cells, with abundant eosinophilic cytoplasm. These proliferating cells were relatively uniform exhibiting round or elliptic nuclei.

Some cases demonstrated the full spectrum of morphologic patterns, ranging from histiocytoid cell populations suspended in a myxoid, edematous stroma with a prominent, delicate vasculature to elongated or spindled cell elements arranged in fascicles occasionally merged with hyalinized areas. In only one case the fascicles concentrically were arranged around vessels. The inflammatory cell infiltrate was composed predominantly of eosinophils, small lymphocytes, plasma cells, macrophages and scattered neutrophils (Figure 1). Hemorrhagic foci were noted in some polyps and hemosiderin was present within macrophages.

**Immunohistochemical findings**

There was a strong diffuse positive staining pattern in the spindle cells with vimentin antibody (Figure 2). A patchy staining for smooth-muscle actin (Figure 3) was observed in these cells. However, it was strongly positive around blood vessels. A heterogeneous reaction was observed with macrophage (HAM-56) antibody. Although immunophenotyping revealed HAM-56-positive cells, their morphological pattern was rather variable. In edematous areas, the HAM-56-positive cells showed voluminous, large cytoplasm and reniform nuclei. In cell-rich areas, the HAM-56-positive cells had fusiform cytoplasm and elongated nuclei (Figure 4). Stains for S-100 and factor VIII-related antigen were negative in the proliferating elements. Factor VIII-related antigen showed positivity in endothelial cells, demonstrating the great vascularity of the lesions. Some remaining nerve fibers were found to be S-100 positive.

**DISCUSSION**

First described in 1949 by VANÉK[28] under the designation of “submucosal granuloma with eosinophilic infiltration”, only a few series of cases of this condition submitted to immunohistochemical studies have been reported[5, 8, 10, 12, 14, 16, 24, 26, 27, 29, 30]. Various names have been used to refer to these rather pleomorphic benign lesions[21], although the term “inflammatory fibroid polyp”, proposed by HELWIG and RANIER[44] has been the most cited. The present study includes one of the larger series in the literature, dealing with clinical and morphological patterns and especially with the immunohistochemical reactivity of spindle/stellate cells, in an attempt to further clarify their histogenesis.

Age, sex and clinical presentation were similar to those recorded in other series[24]. The polyp occurs most commonly in the stomach, generally in the antrum and less frequently in the ileum[9]. Clinical symptoms are variable depending on the location and size of the lesion. As in other reports, abdominal pain and intussusception, the latter almost always found with the small bowel polyp, are among the major symptoms[9].

The lesions are most often found in the gastric antrum (about 70% of cases), followed by the small bowel (about 20% of cases)[9]. Grossly, IPF generally presents as a solitary fibrous, grayish or yellowish-gray mass of spherical or ovoid outline, commonly with ulceration of the
mucosal surface\textsuperscript{(13)}. Intra-lesional hemorrhage, present in some cases, although rarely mentioned, is assumed to be probably due to mechanical trauma in lesions localized in the small bowel with intussusception\textsuperscript{(20)}.

The polyp usually arises from the submucosal region and projects into the lumen of the bowel. The muscle coats may be infiltrated and destroyed\textsuperscript{(9, 13, 29)}. The penetration of the serous layer seen in one of our cases has been described in several papers\textsuperscript{(2, 9, 17, 18, 21, 24, 29)} and has been considered to be of no clinical significance, as demonstrated by the benign course\textsuperscript{(24)}.

At light microscopy, the differences in the amount of collagen fibers and the proportion of infiltrating cells may represent an evolutionary change\textsuperscript{(11, 23)} the highly cellular lesion poor in collagen being the more recent one\textsuperscript{(28)}.

The eosinophilia observed in many lesions has inevitably been a major subject of discussion by many authors. However, such an increase in the number of eosinophils should be interpreted with caution in view of the wide range of conditions in which it may be encountered\textsuperscript{(8, 9, 20)}. In addition, eosinophils can usually be found in moderate number in the normal gastrointestinal mucosa\textsuperscript{(20)}.

Another finding of IFP is the proliferation of small blood vessels. The role of this peculiar vascular structure with elongated cells in concentric layering around vessel has been likened to the pericyte and the pattern has been interpreted to be angiomatous\textsuperscript{(13, 29)}. A previous study has demonstrated the positive peroxidase reaction for muscle actin and “Ulex europeus” in the two main cellular components, supporting the conclusion that IFPs are lesions of vascular origin\textsuperscript{(20)}. However, despite the non-staining of the fusiform cells for endothelial marker, our immunohistochemistry study also showed a rich vascularization of the lesion marked by positivity for factor VIII and smooth muscle actin on the wall of the vessels. These results may indicate a secondary participation of vascular proliferation, if one considers the lesion to be an inflammatory process. However, other authors reported a positivity of the spindle cells for CD 34 and suggested an origin from primitive perivascular or vascular cells\textsuperscript{(30)}.

Recently, a study of 14 cases of IFP showed three cases nonreactive to CD 34. The cases positive for CD 34 had concentric stromal proliferations (CP). The authors concluded that IFP with CP may have different histogenesis from IFP without CP. It was also argued that IFP with CP may originate from a subpopulation of dendritic interstitial cells\textsuperscript{(10)}.

From an ultra structural point of view, the histogenesis of IFP is controversial. The fusiform cells frequently found in the lesion have been considered to be fibroblasts\textsuperscript{(8, 9, 24)} or primitive mesenchymal cells exhibiting incomplete fibrohistiocytic differentiation\textsuperscript{(25, 31)}.

The present immunohistochemical study, like previous ones, has refuted the proposed neuronal or endothelial nature of the lesion, since spindle or stellate proliferating cells were found to be negative for S-100 and factor VII-related antigen\textsuperscript{(4, 5, 8, 14, 16, 24, 25, 29, 30)}. The positivity for lysozyme and alpha 1-anti-trypsin in earlier studies supports the histiocytic\textsuperscript{(11)} and fibrohistiocytic nature of the lesion\textsuperscript{(25)}. However, these are now considered to be rather nonspecific markers and a histiocytic/fibrohistiocytic differentiation was favored in another study since all the 10 cases were found to be positive to CD 68 through the monoclonal antibody KP-1, while factor XIII-related antigen was detected in six (60\%)\textsuperscript{(27)}. In the present study, scattered, spindle-shaped and histiocytoid cells showed positivity to HAM-56 similar to a previous study\textsuperscript{(10)}. These differences may be related to the functional or activated state of the cell, the former resembling fibroblasts and the latter being similar to histiocytes.

Only scattered spindle cells were stained by anti-actin antibody, thus indicating that the cell-rich areas contain at least a few spindle cells, possibly corresponding to myofibroblasts\textsuperscript{(11)}, thus indicating a

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\includegraphics[width=\textwidth]{figure1.png}
\caption{IFP with spindle cells, richly vascularized stroma and inflammatory cells.

H & E X125}
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\begin{figure}[h!]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Diffuse positive staining pattern for vimentin. ABC. X250}
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Spindle cells positive for smooth-muscle actin. ABC. X400}
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\includegraphics[width=\textwidth]{figure4.png}
\caption{HAM - 56 - positive fusiform cells. ABC X250}
\end{figure}
heterogeneous immunophenotype of proliferating cells. Other results confirmed the presence of variable degrees of myofibroblastic histiocytic line of differentiation\[8, 12\].

The strong positivity for vimentin in all cases even in the absence of HAM-56 expression suggests a major component of spindle cells best recognizable as fibroblasts. Each morphological pattern may be considered a phase of an inflammatory reaction, and the presence of inflammatory cells in variable number, especially eosinophils and macrophages, favors this hypothesis.

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