Abstract. Background: We previously reported a novel histological phenotype of chronic esophagitis, lymphocytic esophagitis, in patients without gastroesophageal reflux. The aim of the present study was to explore the possible occurrence of lymphocytic esophagitis in baboons. Materials and Methods: Filed hematoxylin and eosin (H&E)-stained sections from the esophagi of 103 consecutive baboons were reviewed. Lymphocytic esophagitis is characterized by high numbers of intraepithelial lymphocytes (IELs) gathered mainly around papillary areas and by none to occasional CD15+ intraepithelial granulocytes. Results: Forty-five of the 103 baboons (43.7%) had lymphocytic esophagitis. A mean of 52 IELs/high-power field were found around the papillae. Immunostains showed that the IEL population in lymphocytic esophagitis was composed of T-cells, a subset of natural killer cells and of helper and inflammatory T-cells. Conclusion: Since lymphocytic esophagitis is by far much more frequent in baboons than in humans, the baboon emerges as a good animal model to study the etiology of this inflammatory disease in humans.

The esophagus in covered by a multilayer of nonkeratinized stratified squamous cells that protects the underlying tissues from noxious agents and irritants contained in passing solids and fluids (1). Low pH gastric juices refluxed into its lumen provide a major insult to the esophageal epithelium. This gastroesophageal reflux (GER) (2), if persistent, may severely alter the homeostasis of the esophageal microenvironment leading to mucosal inflammation followed, in severe cases, by ulceration and the replacement of deeper layers by fibrotic tissue (3). The sequence of these events is known as gastroesophageal reflux disease (GERD) (4).

Inflammatory changes in the esophagus may also be induced by other external factors such as bacteria, viruses, fungi (Candida albicans), radiation, caustic substances, corticoid or antibiotic therapy and by diseases such as diabetes (2, 5). Acid reflux from the stomach, however, is the most common cause leading to inflammation.

In reflux esophagitis, the histological changes occurring in the squamous epithelium of the esophagus have been systematized by Ismail-Beiji and Pope (5). Similar histological changes were recently found in 8 baboons having reflux esophagitis (6).

Years ago, while reviewing the histology of the stomach in baboons (7), we noticed in one that the esophageal epithelium was infiltrated by a large number of lymphocytes. Looking for similar changes in the esophagus of humans, we recently found such lymphocytic esophagitis in the esophageal biopsies of 20 patients (8). In none of these 20 patients could gastroesophageal reflux be demonstrated as the cause of this. Lymphocytic esophagitis is characterized by a high number of intraepithelial lymphocytes (IELs) mainly in the epithelium around the papillae (which is centred by a papillary vessel) and less prominent in the epithelium between the papillary folds. There is absence of granulocytic (neutrophils and/or eosinophils) infiltration (8). The papillary folds are usually taller than normal but lack the basal cell hyperplasia seen in reflux esophagitis (5, 6).

We recently reviewed the esophagi in non-human primates (NHP) aiming to explore the frequency of lymphocytic esophagitis.

Materials and Methods

A total of 103 consecutive esophagi (sampled at autopsy) were collected from olive or olive/yellow hybrid baboons (Papio hamadryas anubis, Ph. cynocephalus).

The baboons were members of colonies at the Southwest National Primate Research Center, Southwest Foundation for Biomedical Research. The conditions of animal housing have been reported elsewhere (6). Briefly the baboons were housed in metal and concrete indoor-outdoor cages and were fed commercial...
monkey diets occasionally supplemented with a variety of fruit and vegetables. Water was available *ad libitum*.

The animals were euthanized with a commercial barbiturate agent because of advanced non-GI diseases or natural causes such as ageing. All procedures were carried out in accordance with the Institutional Animal Care and Use Committee guidelines.

At necropsy, longitudinal tissue samples from the esophagus were fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 μm, stained with hematoxylin and eosin (H&E) and evaluated under light microscopy.

Immunohistochemistry was performed in 4 animals with lymphocytic esophagitis using CD3 (dilution 1:400; Neo Markers, Inc, Freemont, CA, USA) to label T-lymphocytes, CD2 (dilution 1:60; Thermo Fisher Scientific, Freemont, CA, USA) to label T-cells and a subset of natural killer cells and CD4 (dilution 1:20; Novocastra, New Castle upon Tyne, UK) to label helper and inflammatory T-cells.

Counting of IELs was performed at ×20 magnifications in H&E-stained sections, in the most affected epithelial areas, without knowledge of the clinical data of the 103 baboons. The expression of these immunostains was graded as 0: no expression, +: weak expression, ++: moderate expression and +++: marked expression.

**Results**

The histological characteristics of the normal esophagus and of lymphocytic esophagitis in baboons are shown in Figures 1-3.

*Lymphocytic esophagitis.* The counting of intraepithelial lymphocytes in H&E sections showed that of the 103 consecutive esophagi examined in baboons, 43.7% (45/103) had lymphocytic esophagitis. A mean of 52 IELs /high-power (HP) field (range 38 to 58 IELs /HP field) were found around the papillae of the squamous epithelium. A much lower lymphocytic infiltration (mean 12 IELs /HP field, range 9 to 28 IELs /HP field) was found in the epithelium between papillary folds.

Immunostaining showed that the IELs in lymphocytic esophagitis were CD2+++ (Figure 4), CD3+++ and CD 4++.

**Discussion**

Nearly 45% of the esophagi investigated in baboons, euthanized for reasons other than gastrointestinal symptoms, had lymphocytic esophagitis at histological examination.

The IELs in the animals with lymphocytic esophagitis were found in the epithelium around the papillae (centered by a papillary vessel) and less frequently, even in the epithelium between the papillary folds. After crossing
through the papillary vessel, IELs apparently spread laterally within the squamous epithelium in some animals. The number and distribution of IELs in lymphocytic esophagitis in baboons and in humans (8) contrasts with that in reflux esophagitis in humans (5) and in baboons (6). In lymphocytic esophagitis the number of IELs was significantly higher than in reflux esophagitis (8). Whereas in lymphocytic esophagitis, the IELs are found mainly around the papillary areas, in reflux esophagitis the IELs are particularly found between the papillary folds (8). These findings suggest that in lymphocytic esophagitis, the bulk of the IELs might have traversed the basement membrane at the level of the papillary folds of the squamous epithelium (8).

The main histological criteria for diagnosing lymphocytic esophagitis in baboons were the presence of a high number of IELs and the absence of granulocytes (8). In contrast, in grade 2 and 3 reflux esophagitis, many intraepithelial granulocytes (neutrophils and/or eosinophils) were seen within the epithelium (6, 8).

In lymphocytic esophagitis, immunohistochemistry showed that the IEL population was composed of T-cells, a subset of natural killer cells and of helper and inflammatory T-cells.

The antigen(s) responsible for the marked immunological reaction in the squamous epithelium of the esophagus in lymphocytic esophagitis is at present unknown. It is also enigmatic why lymphocytic esophagitis is so frequent in baboons and rather uncommon in humans (8). This difference between species raises intriguing questions: a) Does the oblique position often adopted by baboons play a role in the development of lymphocytic esophagitis? b) Does the diet trigger the initiation of the disease in baboons? c) Are other environmental pollutants decisive for the occurrence of the disease in baboons? d) Does the stress (9) of these animals kept in captivity contribute to the development of lymphocytic esophagitis? In this respect it should be remembered that the cotton-top tamarin Sanguinis oedipus oedipus, a marmoset species native to a small region in Colombia, often develops a diffuse inflammation in the colonic mucosa that resembles ulcerative colitis in humans. Interestingly, these inflammatory changes only develop when the cotton-top tamarins are kept in captivity.

One possibility to explore the effect of the environment on baboons would be to assess the frequency of lymphocytic esophagitis at other facilities engaged in primate research. The use of different food regimens at other facilities would identify whether the diet has any bearing in the triggering of lymphocytic esophagitis in baboons.

Since lymphocytic esophagitis is by far much more frequent in baboons than in humans, the baboon emerges as a good animal model to study the etiology of this inflammatory disease in humans.

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