Morphological differences in epithelial papillae and microvascular architecture according to site in cat filiform papillae

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There have been no reports comparing the morphologies of epithelial papillae (EP) and the microvascular architecture of the cat filiform papillae (FP). We observed and compared the morphologies of EP and the microvascular architecture of FP at various locations under a scanning electron microscope. We found that EP were composed of a base and processes, which were divided into a main process and accessory processes. The accessory processes existed only in the anterior half of the tongue. Based on the shapes of the main process and arrangements of the accessory processes, we classified FP into the following five types: circular conical, complex conical, complex columnar, simple columnar, and simple conical. The microvascular architecture was composed of a capillary plexus and multiple capillary loops (CLs). The capillary plexus was classified as either conical or columnar. The CLs were arranged in an ellipse for the conical type, and in a semicircle for the columnar type. Based on the comparison of EP and microvascular architecture, the morphology of the main process matched that of the capillary plexus throughout. In contrast, no correspondence whatsoever was observed between the number of accessory processes and CLs. (J Osaka Dent Univ 2015; 49(1): 35–48)

Key words: Filiform papillae; Epithelial papillae; Microvascular corrosion cast; Tongue; Cat

INTRODUCTION

Regarding cat filiform papillae (FP), Kobayashi et al. (1988, 1992) and Ojima et al. (1996, 2000) reported on the epithelial papillae (EP) and microvascular architecture, respectively. Both noted that the morphology of the FP differed according to location. However, the morphologies of EP and the microvascular architecture have not been compared for the same site. We used a scanning electron microscope (SEM) to observe the EP and microvascular architecture of the FP in each site of the dorsum of the feline tongue, and compared the morphologies of the EP and the microvascular architecture in each site. We were able to obtain novel findings regarding EP and the microvascular architecture.

MATERIALS AND METHODS

The animal subjects were four male adult cats (Felis catus) with an average body weight of about 3.0 kg (Hamaguchi Laboratory Animals, Hyogo, Japan). After anesthesia was induced with inhalation of isoflurane (Forane®; Abbott Japan, Tokyo, Japan), the cats were intraperitoneally injected with 5,000 units of heparin sodium (Novo Heparin Injection 5000®; Mochida Pharmaceutical, Tokyo, Japan). After 30 minutes, the cats were euthanized with overdoses of intraperitoneally administered pentobarbital (Nembutal®; Dainippon Sumitomo Pharma, Osaka, Japan). After decapitating two of the cats, we inserted cannulae in the left and right common carotid arteries to observe the EP. Then, using an infusion pump (PST-1000®; Iwaki, Tokyo, Japan), we performed perfusion fixation by infusing both arteries with a saline solution containing 2.0% paraformaldehyde and 2.5% glutaraldehyde (both from Kishida Chemical, Osaka, Japan). After resecting the tongues, we cut a sagittal sec-
tion of one of the two tongues, and divided one side
frontally into four equal parts (Fig. 1). We macroscopi-
cally observed the other tongue, and then post-fixed
both of them by soaking in the above-mentioned solu-
tion at 4°C for 24 hours. After washing the two re-
sected tongues with 0.1 M phosphate buffer solution,
we performed conductive staining by soaking them in
1% tannic acid and 2% osmic acid (both from Kishida
Chemical) for four hours each. We then dehydrated
the tongues with ascending alcohol solutions, which
were subsequently replaced with tert-butyl alcohol.
Next, we lyophilized the tongues using a lyophilizer
(JFD-310®; JEOL, Tokyo, Japan). After fixing the
specimens to the stage using conductive tape (NEM
Tape®; Nisshin EM, Tokyo, Japan) and silver paste
(Dotite®; Fujikura Kasei, Tochigi, Japan), we ob-
served the specimens with a SEM (JSM-5500®; JE-
OL).

When observing FP, as shown in Fig. 1, we divided
the length of the tongue body from anterior to poste-
rior into four equal parts, the lingual apex, anterior,
middle and posterior regions. We defined the poste-
rior end of the tongue body as the posterior margin of
the vallate papillae, located in the most posterior part
of the tongue body. In measuring the length of the
main process (M) of the FP, we arbitrarily selected 5
FP in each portion in the dorsal midline area of one
tongue. Image analysis was done using a SEM image
of the superior view of the FP image. As shown in
Fig. 2, we measured the length of the M using imaging
analysis software (Image-Pro Plus® 5.1 J; Nippon
Roper, Tokyo, Japan). Measurements are repre-
sented as the mean and standard deviation. We also
counted the numbers of accessory processes.

As for the remaining two cats, we used the method
described by Suwa et al. (2013) to create microvas-
cular corrosion cast specimens. After decapitating
the cats, we inserted cannulae into the left and right com-
mon carotid arteries. Then, using an infusion pump,
we perfused both arteries with 1.0 L saline solution to
remove the blood from the internal jugular vein. Next,
using a precise syringe pump (KDS 200 P®; Muroma-
chi Kikai, Tokyo, Japan), we injected low- and high-
viscosity acrylic resins into both carotid arteries. We
injected 400 mL of the low-viscosity acrylic resin at a
rate of 8 mL/min, and 30 mL of the high-viscosity
acrylic resin at a rate of 2 mL/min. To maintain the dis-
tance between the maxilla and mandible, we mounted
plastic tubes 1 cm in length and 6 mm in diameter be-
tween the maxillary and mandibular first molars.

After the resin polymerized, we resected the
tongues and created microvascular cast corrosion
specimens. We cut one of the two tongues in a sagittal
direction, and one side was divided frontally into four equal parts (Fig. 1). To do so, we soaked the two tongues in 10% sodium hydroxide solution for 48 hours at 48°C. Next, we replaced this solution, soaked the tongues again for 48 hours, removed the soft tissue, washed the tongues with running warm water (40°C), and then air-dried them. These specimens were then fixed to a stage using conductive tape and silver paste. After using an ion-sputter coating device (JFC-1500; JEOL) to coat gold, we observed the specimens under SEM.

This animal study was approved by the Osaka Dental University Animal Research Committee (Approval numbers 12−10003, 13−02032, and 14−03012) and performed in accordance with the guidelines related to animal experiments.

RESULTS

Macroscopic observation

After fixation, the mean anteroposterior length of the cat tongues from the lingual apex to the anterior base of the epiglottis was approximately 54 mm; the width at the lingual apex, approximately 16 mm; and the width from the anterior region to the root of the tongue, approximately 20 mm. The shape of the tongue was rectangular, with the lingual apex region in a U-shape. The dorsum of the tongue was flat. The marginal portion of the tongue from the lingual apex to the anterior region was thin, and the central portions of the anterior, middle and posterior regions were thick. The midline groove of the tongue was indistinct. The terminal sulcus of the tongue could not be confirmed. In addition, in the vicinity of the midline of the boundary between the middle and posterior regions, a macroscopically distinguishable boundary with a distinct, nearly-pear shape was observed. We defined this area as the island portion (Fig. 1).

Scanning electron microscopy

Epithelial papillae (EP)

FP were present throughout the dorsum of the tongue. The shape of the filiform papilla was basically composed of a base and processes (Fig. 3). The shapes of the base were either an elliptic cylinder or columnar. The processes protruded from the superior surface of the base. The FP bases and processes demonstrated different morphologies in each region. A process that was the thickest, longest, and usually inclined in the direction of the pharynx was defined as the main process (M), while all the other processes were defined as accessory processes (APs). When there was only one process, it was defined as the M. The shape of the M was either conical or columnar, whereas the APs were only conical.

Based on the shape and arrangement of the processes, we defined five types of FP (Fig. 4) as follows: 1) FP with a single conical M and 6–10 APs surrounding the circumference of the superior surface of the base were defined as circular conical FP; 2) FP composed of a single conical M and 2–4 APs were defined as complex conical FP; 3) FP composed of a single giant columnar M and 2–4 APs were defined as complex columnar FP; 4) FP composed of only a single giant columnar M were defined as simple columnar FP; 5) FP composed of only a single conical M were defined as simple conical FP.

Based on the distributions of the five above-mentioned types of FP observed in the four regions of the tongue that were defined earlier, we subdivided...
each portion as follows: the lingual apex region was divided into the marginal, intermediate and anterior tongue portions; the anterior region was divided into the marginal, intermediate, anterior tongue and central portions; the middle region was divided into the central, posterior tongue and island portions; and the posterior region was divided into the island and posterior tongue portions. These portions and the types of FP distributed in them are shown in Fig. 4. Detailed observation findings regarding the FP bases and processes in the above-mentioned regions are described in the following section. Hereafter, FP are described as EP.

**Lingual apex region**

**Marginal portion of the lingual apex**

The FP in this portion were the circular conical type (Fig. 5A). The EP were composed of an elliptic cylindrical base, a single conical M of length $245 \pm 35 \mu m$ surrounding the circumference of the superior surface of the base in an elliptical shape, and 6–10 conical APs. The APs protruded dorsally.

**Intermediate portion of the lingual apex**

The FP in this portion were the complex conical type (Fig. 5B). The EP were composed of an elliptic cylindrical base, a single conical M of length $527 \pm 81 \mu m$, and 2–4 conical APs. The APs protruded dorsally.
Anterior tongue portion of the lingual apex
The FP in this portion were the complex columnar type (Fig. 5 C). The EP were composed of a columnar base, a single columnar M of length $1354 \pm 89 \, \mu m$, and 2−4 conical APs. The APs protruded in the direction of the pharynx.

Anterior region
Marginal portion of the anterior region
The FP in this portion were the circular conical type (Fig. 6 A). The EP were composed of an elliptic cylindrical base, a single conical M surrounding the circumference of the superior surface of the base in an elliptical shape, and 6−10 conical APs. The APs protruded dorsally.

Intermediate portion of the anterior region
The FP in this portion were the complex conical type (Fig. 6 B). The EP were composed of an elliptic cylindrical base, a single conical M protruding from the su-

Fig. 6 Superior view of anterior region. (A) In the marginal portion, the filiform papillae are the circular conical type. Processes are composed of a conical main process (M) and six accessory processes (APs, arrows) arrange in an ellipse. (B) In the intermediate portion, the filiform papillae are the complex conical type. Processes are composed of a long M and four APs (Ba : Base). (C) In the anterior tongue portion, the filiform papillae are the complex columnar type. Processes are composed of a long M and four APs. (D) In the central portion, the filiform papillae are the simple columnar type. (E) In the posterior view of the central portion, depressions (asterisks) are observed in the M on the posterior surfaces.
prior surface of the base, and 2−4 conical APs. The APs protruded dorsally.

**Anterior tongue portion of the anterior region**

The FP in this portion were the complex columnar type (Fig. 6 C). The EP were composed of a columnar base, a single columnar M protruding from the superrior surface of the base, and 2−4 conical APs. The APs protruded in the direction of the pharynx.

**Central portion of the anterior region**

The FP in this portion were the giant, simple columnar type (Fig. 6 D). The EP were composed of a columnar base and a single columnar M of length $1511 \pm 112 \mu m$. The M narrowed as it progressed from the base to the tip, where it was rounded. A depression was observed in the M on its posterior surface (Fig. 6 E).

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**Fig. 7** Superior view of middle region. (A) In the central portion, the filiform papillae are the simple columnar type. (B) In the posterior tongue portion, the filiform papillae are the simple conical type. (C) In the island portion, the filiform papillae are the simple conical type.

**Fig. 8** Superior view of posterior region. (A) In the island portion, the filiform papillae are the simple conical type. (B) In the posterior tongue portion, the filiform papillae are the simple conical type.
Middle region

Central portion of the middle region
The FP in this portion were the simple columnar type (Fig. 7 A). The EP were composed of a columnar base and a single columnar M of length $1574 \pm 54 \, \mu m$.

Posterior tongue portion of the middle region
The FP in this portion were the simple conical type (Fig. 7 B). The EP were composed of an elliptic cylindrical base and a single conical M of length $745 \pm 20 \, \mu m$.

Island portion of the middle region
The FP in this portion were the simple conical type (Fig. 7 C). The EP were composed of an elliptic cylindrical base and a single conical M of length $302 \pm 36 \, \mu m$.

Posterior region

Island portion of the posterior region
The FP in this portion were the simple conical type (Fig. 8 A). The EP were composed of an elliptic cylindrical base and a single conical M of length $325 \pm 26 \, \mu m$.

Posterior tongue portion of the posterior region
The FP in this portion were the simple conical type (Fig. 8 B). The EP were composed of an elliptic cylindrical base and a single conical M of length $496 \pm 152 \, \mu m$.

Microvascular Architecture

The basic morphology of the microvascular architecture in the FP that were observed in the dorsum of the feline tongue was composed of capillary loops (CLs) and a capillary plexus (P). The arteriole arising in the direction of the dorsum of the tongue from the dorsal lingual branch of the deep lingual artery divided into 4 or 5 branches. After penetrating the mesh of the venular network (Fig. 9), these branches became the ascending crura of the capillaries. The ascending crura transitioned into the descending crura and the latter flowed into the venular network. Each capillary loop (CL) was formed into a hairpin shape by one ascending crus and one or a few descending crura. In each FP, 4–26 CLs were observed. Each P was a capillary network formed three-dimensionally by multiple ascending and descending crura. Two types of P (conical and columnar) were observed. Each conical P had a CL observed at its vertex. Each columnar P had a round tip but no CL. One P was observed in each FP. Each P was located mostly in the direction of the pharynx, inclined in the direction of the pharynx, and longer than any CL. In accordance with the categories based on observational findings in EP, the microvascular architecture in the FP in each portion is described in the following section.

Linguapapillae region

Marginal portion of the lingual apex region
The microvascular architecture in this portion was composed of 4–9 CLs and a single conical P (Fig. 10 A). The CLs and P were arranged in a linguapapillary direction, in the shape of a long ellipse. The CLs protruded dorsally.
Intermediate portion of the lingual apex
The microvascular architecture in this portion was composed of 10–14 CLs and a single conical P (Fig. 10 B). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally and dorsal-laterally.

Anterior tongue portion of the lingual apex
The microvascular architecture in this portion was composed of 12–22 CLs and a single columnar P (Fig. 10 C). The CLs, which were gathered in front of the P (i.e., in the direction of the lingual apex), were arranged in a semicircle. The CLs protruded dorsal-laterally along the circumference of the semicircle.

Anterior region
Marginal portion of the anterior region
The microvascular architecture in this portion was composed of 4–6 CLs and a single conical P (Fig. 11 A). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally.

Intermediate portion of the anterior region
The microvascular architecture in this portion was composed of 10–14 CLs and a single conical P (Fig. 11 B). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally.

Anterior tongue portion of the anterior region
The microvascular architecture in this portion was composed of 12–26 CLs and a single columnar P (Fig. 11 C). The CLs, which were gathered in front of the P (i.e., in the direction of the lingual apex), were arranged in a semicircle. The CLs protruded dorsal-laterally along the circumference of the semicircle.
Central portion of the anterior region

The microvascular architecture in this portion was composed of 15–25 CLs and a single columnar P (Fig. 11 D). The CLs, which were gathered in front of the P (i.e., in the direction of the lingual apex), were arranged in a semicircle. The CLs protruded dorsolaterally along the circumference of the semicircle. The P gradually narrowed toward the tip and was rounded at the tip. In addition, the posterior surface of the P was concave (Fig. 11 E).

Middle region

Central portion of the middle region

The microvascular architecture in this portion was
composed of 12–16 CLs and a single columnar P (Fig. 12 A). The CLs, which were gathered in front of the P (i.e., in the direction of the lingual apex), were arranged in a semicircle. The CLs protruded dorsolaterally along the circumference of the semicircle.

**Posterior tongue portion of the middle region**
The microvascular architecture in this portion was composed of 7–13 CLs and a single conical P (Fig. 12 B). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of an ellipse. The CLs protruded dorsally.

**Island portion of the middle region**
The microvascular architecture in this portion was composed of 10–14 CLs and a single conical P (Fig. 12 C). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally.

**Posterior region**

**Island portion of the posterior region**
The microvascular architecture in this portion was composed of 10–12 CLs and a single conical P (Fig. 13 A). The CLs and P were arranged in a lingual
apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally.

**Posterior tongue portion of the posterior region**
The microvascular architecture in this portion was composed of 10–16 CLs and a single conical P (Fig. 13 B). The CLs and P were arranged in a lingual apex-pharynx direction, in the shape of a long ellipse. The CLs protruded dorsally.

**DISCUSSION**

**Epithelial papillae**

**Classification of EP**

Kobayashi et al. (1988, 1992)\(^1,2\) divided cat FP into four types, namely large, small, intermediate, and giant conical. However, they did not state anything regarding the morphology of intermediate FP. Based on the shapes and arrangements of the M and AP, we divided FP into five types as follows: circular conical, complex conical, complex columnar, simple columnar, and simple conical (Fig. 4). The large FP observed by Kobayashi et al. seem to correspond to the simple columnar FP in our study. In addition, the small FP that they observed seem to correspond to our circular conical FP, while their giant conical FP seem to correspond to our simple conical FP. Kobayashi et al. stated nothing about FP corresponding to the complex conical and complex columnar FP that we observed. Therefore, complex conical and columnar FP, which were not reported by them, should be added to the categories of cat FP.

**Morphological changes in EP according to site**

**Changes in M length**

In order to compare M lengths from the anterior to the posterior dorsal tongue midline area, we assembled the M lengths of all the EP observed in all portions in the midline area into a graph (Fig. 14). The M increased in length from the marginal portion of the lingual apex, to the intermediate portion of the lingual apex, to the anterior tongue portion of the lingual apex, and then on to the central portion of the anterior region. The M was longest in the central portion of the middle region. It decreased in length from the central portion of the middle region, to the posterior tongue portion of the middle region, and on to the island portion of the middle region. The M was shortest in the is-
land portion of the middle region (Fig. 14), and was slightly longer in the posterior region than in the island portion of the middle region. The FP functions to retain food before swallowing. Thus, this rapid increase in the length of the M from the lingual apex region to the central portion of the middle region may play a role in food retention.

Number of AP
We made a list of the number of AP in all portions of the tongue (Table 1) based on the diagram in Fig. 4. In doing this, we found that the largest number of AP existed in the marginal portion of both the lingual apex and anterior regions. Meanwhile, a few APs existed in the intermediate and anterior tongue portions of both regions. When there were large numbers of APs, space was created among them, and water was trapped in these spaces by capillary action. We believe that this facilitates drinking water.

Comparisons of the M and AP of cats with those of other carnivores
We found only five reports on the lingual papillae of cats, which are carnivores of the family Felidae. These reports did not list numerical values such as the length or number of FP processes; therefore, their results could not be used for comparisons. However, a report on ferrets, which are carnivores of the family Mustelidae, did list such numerical values. In this report on ferrets, the M grew longer toward the posterior region of the tongue. APs were observed in the lingual apex, anterior and middle regions, but not in the posterior region. The number of APs decreased toward the posterior end of the tongue. Thus, while the M was longest in the central portion of the middle region in cats, it was longest in the posterior region in ferrets. In cats, APs ceased appearing in the central portion of the anterior region. In ferrets, APs were not observed in the posterior region.

Microvascular architecture
Microvascular architecture terminology
Ojima et al. (1996, 2000) divided the microvascular architecture of FP into five categories as follows: types I – III, in which both the M and AP are present, and types IV and V, which contain no M and are composed only of APs. The terms M and AP used by Ojima et al. are used only for EP and should not be used for microvascular architecture. Therefore, we propose the use of the terms P and CL to describe the microvascular architecture of FP.

Classification of microvascular architecture
Accordingly, in our discussion, for easier comparison.

### Table 1 The shape of epithelial papillae, the shape of the capillary plexus, the number of accessory processes, and the number of capillary loops in a filiform papilla

<table>
<thead>
<tr>
<th>Region</th>
<th>Portion</th>
<th>Epithelial papilla</th>
<th>Microvascular architecture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shape of filiform papilla</td>
<td>Number of accessory processes</td>
</tr>
<tr>
<td>Posterior</td>
<td>Posterior tongue</td>
<td>Simple conical</td>
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</tr>
<tr>
<td></td>
<td>Island</td>
<td>Simple conical</td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>Posterior tongue</td>
<td>Simple conical</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>Simple columnar</td>
<td>2–4</td>
</tr>
<tr>
<td>Anterior</td>
<td>Central</td>
<td>Simple columnar</td>
<td>0</td>
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<tr>
<td></td>
<td>Intermediate</td>
<td>Complex conical</td>
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</tr>
<tr>
<td></td>
<td>Marginal</td>
<td>Circular conical</td>
<td>2–4</td>
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</tbody>
</table>

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of the categories established by Ojima et al. and those set by us, we replaced their M and AP with our P and CL, respectively. They referred to types IV and V, in which there were no P. However, we observed the P in all sites on the dorsum of the tongue; thus, no microvascular architecture lacked a P, as reported by Ojima et al. Therefore, we focused on the morphology of the P, dividing it into two categories (conical and columnar); by doing so, we were able to simplify the classifications.

**Changes in the number of CLs according to site**
To compare the number of CLs from the anterior to the posterior in the dorsal tongue midline, we assembled the number of CLs observed in all portions (Table 1). The number of CLs increased from the marginal to the intermediate portion of the lingual apex, the anterior tongue portion of the lingual apex, and then to the central portion of the anterior region, where the number of CLs was highest. The number of CLs decreased from the central portion to the posterior tongue portion of the middle region. The island portion of the middle and posterior regions demonstrated slightly higher numbers of CLs than the posterior tongue portion of the middle region.

**CLs in the central portion of the anterior region**
The central portion of the anterior region is where the number of CLs is highest. In their comparative observations of EP and lamina propria papillae, Kobayashi et al. (1988) stated the following, “The architecture in the epithelium which contains lamina propria papillae is considered to constitute a mechanical structure that can serve as either the foundation of the posterior M or as its support.” The P and CLs are found within lamina propria papillae; the P in the M; and CLs in the base. Therefore, the P is thought to mechanically support the M, whereas CLs are thought to mechanically support the P. Based on the aforementioned, the CLs mechanically support the P in the central portion of the anterior region, where the number of CLs must also be higher than that in any other site.

**Comparisons of the P and CLs of cats with those of other carnivores**
The only detailed description of microvascular architecture that we could find was in ferrets, which are carnivores of the family Mustelidae. Therefore, we compared the microvascular architectures in ferrets and cats. The P observed in cats was not found in ferrets. Although cats demonstrated differences in the numbers of CLs according to site, almost no such difference based on site was observed in ferrets. Based on the aforementioned, the major differences in microvascular architecture between cats and ferrets were that the P was observed in cats but not in ferrets, and that cats demonstrated differences in the number of CLs according to site.

**Comparisons of epithelial papillae and microvascular architecture morphologies**

**Comparison of M and P morphologies**
When M presented a conical morphology, the morphology of the P was always conical, and when the M presented a columnar morphology, the morphology of the P was always columnar (Table 1). Therefore, the morphology of the P matched the morphology of the M, covering the periphery of the P.

**Comparison of the number of APs and CLs**
The numbers of APs and CLs in each portion are summarized in Table 1. APs were not observed in the central portion of the anterior region, nor in any portion of the middle or posterior regions. Therefore, we could not make comparisons for these sites. In the lingual apex region, where APs were observed, the number of APs decreased from the marginal to the intermediate and anterior tongue portions. However, the number of CLs in the lingual apex region increased from the marginal to the intermediate portion and then to the anterior tongue portion (Table 1). Therefore, no correspondence whatsoever was observed between the number of APs and number of CLs. Based on the aforementioned, we think that CL is absent in the AP, but present in the base.

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