Spontaneous T-cell-rich B-cell Lymphoma in a Cynomolgus Monkey (Macaca fascicularis)

Masaki MICHISHITA1), Shin-ichiro NAKAMURA1), Ippei SAKAKIBARA2), Fumiko ONO3), Kouji FUJIMOTO3), Kazusaku KAMIYA4), Yoshiyuki ISHI4), Kazuhiko HAYASHI5), Yasuhiro YOSHIKAWA4), and Kimimasa TAKAHASHI1)

1)Department of Veterinary Pathology, Nippon Veterinary Animal and Science University, 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180-8602, 2)Tsukuba Primate Center for Medical Science, National Institute of Infectious diseases, 1 Hachimandai, Tsukuba-shi, Ibaraki 305-0843, 3)The Corporation for Production and Research of Laboratory Primates, 1 Hachimandai, Tsukuba-shi, Ibaraki 305-0843, 4)Department of Biomedical Science, Graduate School of Agricultural and Life Science, University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113-8657, and 5)Second Department of Pathology, Okayama University Medical School, 2–5–1 Shikata-cho, Okayama-shi, Okayama 700-8558, Japan

Abstract: A spontaneous T-cell-rich B-cell lymphoma (TCRBCL) occurred as a subcutaneous mass in the buccal region and enlarged submandibular lymph node in a 6-year-old female cynomolgus monkey (Macaca fascicularis). The constituent cells were examined by histology, immunohistochemistry and the double labeled-immunofluorescence method (dl-IF). Further, in situ hybridization (ISH) was employed to detect the gene expression of Epstein Barr virus (EBV). Histologically, the mass was comprised mainly of neoplastic large lymphoid cells and reactive small mononuclear cells. Immunohistochemically, the neoplastic large lymphoid cells were positive for CD20, CD79α, MHC class II, and either IgG, IgM, or IgA. Polyclonal Ig production by the neoplastic large lymphoid cells was demonstrated by dl-IF, although IgG-positive ones predominated in number. On the other hand, most of the small mononuclear cells were positive for CD3 and were regarded as reactive T lymphocytes, while the remaining cells appeared to be histocytes or reactive B-cells. Transcripts of EBV gene were not demonstrated in these neoplastic or reactive cells by ISH. This is the first reported case of spontaneous TCRBCL in the cynomolgus monkey.

Key words: cynomolgus monkey, spontaneous, T-cell-rich B-cell lymphoma

Spontaneous lymphomas have infrequently been reported in nonhuman primates, such as chimpanzees, African green monkeys, gibbons, baboons, cynomolgus monkeys, and rhesus monkeys [1, 4, 7, 8, 12, 14–16, 22, 24]. Simian T-cell lymphomas, which are often associated with simian T-cell leukemia virus, have been found in baboons and African green monkey [13, 22], while simian B-cell lymphomas have been reported in
rhesus monkeys [24]. Although various primate species are latently infected with Epstein Barr virus (EBV) [9] that causes a juvenile type of B-cell lymphoma (Burkitt lymphoma) in humans, there has been no report on spontaneous lymphomas associated with EBV in those species.

T-cell-rich B-cell lymphoma (TCRBCL) has been characterized as a morphological variant of the diffuse B-cell lymphoma group and its criteria is a large B-cell tumor with reactive T-cells occupying more than 50% of the cellular population [19]. Recently, TCRBCL has been reported not only in humans [3, 20, 21] but also in some nonhuman species [2, 6, 17, 23, 26]. Although it has been reported that EBV was associated with the pathogenesis of TCRBCL in humans [25, 27], such a phenomenon has not been seen in other animal species.

In the present study, spontaneous TCRBCL which occurred in the buccal region of a 3-year and 9-month-old female cynomolgus monkey was studied by histological, immunohistochemical and double-labeled immunofluorescence (dl-IF) examinations. Furthermore, the correlation between TCRBCL and EBV was examined by an in situ hybridization (ISH) method.

The mass was first noted in the subcutis of the buccal region of a 3-year and 9-month-old female cynomolgus monkey (Macaca fascicularis) being kept for breeding at the Tsukuba Primate Center for Medical Science, National Institute of Infectious Diseases, Japan. X-ray examination showed an osteolytic pattern of the maxillary bone surrounding the mass. Thereafter, in addition to the mass, an enlarged submandibular lymph node was found and surgically removed when the animal was 5 years and 9 months old. However, the complete removal of the buccal mass was difficult because it was tightly attached to the adjacent tissue. The monkey had clinically poor appetite and diarrhea. Hematology and blood biochemistry revealed leukocytosis and high levels of lactate dehydrogenase. Serologically, a positive antibody reaction against EBV was shown. After surgical removal, the tumors recurred in the same portions. The animal was euthanatized because of poor prognosis when she was 6 years old.

At necropsy, the primary buccal mass and enlarged mandibular lymph node were about 7 cm in diameter, respectively. The cut surfaces were homogeneously grayish white. No metastasis of the tumor to other organs was confirmed macroscopically.

Tissue samples were fixed in 20% neutral buffered formalin, embedded in paraffin, and sectioned 3 µm in thickness. Deparaffinized sections were stained with hematoxylin and eosin (HE), and further subjected to other stains as necessary.

Some deparaffinized sections were used for immunohistochemistry. Primary antibodies used in the present study were monoclonal antibodies against human CD20, CD79α, MHC class II (HLA-DR), HAM56, MAC387 and swine vimentin, and polyclonal antisera against human CD3, IgG, IgA, IgM, lysozyme and C3c complement (Table 1). Antisera against human CD3, IgG, and lysozyme are known to be also applicable to primates [12]. For detecting some antigens, sections were pretreated with 1% trypsin and with subsequent hydrated autoclave or microwave methods. Secondary antibodies used were biotinylated goat antisera (DAKO, Denmark) against mouse or rabbit immunoglobulin (Ig). These sections were reacted with peroxidase-conjugated streptavidin (LSAB, DAKO) and visualized with 3,3′-diaminobenzidine tetrahydrochloride (Dojindo, Japan) plus hydrogen peroxidase. The mean value (%) of CD3-positive small mononuclear cells per whole cellular population was determined by counting microscopically in at least 7 different fields of 400X magnification.

For the double-labeled immunofluorescence method, a combination of an indirect method with mouse monoclonal antibody to human IgG (DAKO, Denmark) and Texas red-conjugated goat antisera to mouse IgG (Vector, USA) and a direct method with FITC-conjugated polyclonal antisera against human IgA or IgM (DAKO, Denmark) was employed. Stained sections were observed under a confocal laser microscope (Laser Scanning Microscope 510, Carl Zeiss, Germany).

To detect EBV-encoded small RNA-1 (EBER-1), ISH was employed using single-stranded FITC-labeled 30-base oligonucleotide probes, complementary (antisense probe, 5′-AGACACCCGTCCTCACACCACCGGGACTTGTA-3′) or anticomplementary (sense, negative control probe) [5]. Formalin-fixed, paraffin-embedded sections from the buccal mass and mandibular lymph node were hybridized with the sense or antisense probe. They were further visualized using a commercialized in situ hybridization kit (DAKO, Denmark), as described previously [11].

The tumor was composed of two types of cells,
namely neoplastic large lymphoid cells and well-differentiated small mononuclear cells, both of which were distributed diffusely (Fig. 1). The neoplastic large lymphoid cells were relatively pleomorphic in shape, and had slightly eosinophilic, abundant cytoplasm with frequent vacuoles. They had a large, hypochromatic nucleus with plural large nucleoli. Some of these cells contained PAS-positive substances in the perinuclear area. Neoplastic multinucleated giant cells were occasionally observed (Fig. 2). On the other hand, the small mononuclear cells showed oval or elongated shape, and had scant cytoplasm. Their nuclei were round or oval, and uniformly hyperchromatic. Neither nuclear atypia nor mitotic figure was observed in the small mononuclear cells. The submandibular lymph node also showed histopathological changes similar to the buccal mass. The original structure of the lymph node was almost destroyed by proliferation of these tumor cells. Histopathological findings of the mass excised surgically while alive were similar to those of the tumor obtained at necropsy.

Both neoplastic large lymphoid cells and multinucleated giant cells were positive for CD20 (Fig. 3), CD79α, IgG (Fig. 4), IgA, IgM, and vimentin (date not shown), but negative for CD3. Thus, these cells exhibited the character of B lymphocyte with various Ig production. Furthermore they were slightly positive for HLA-DR (Fig. 5). A large number of the small mononuclear cells were, in contrast, positive for CD3 (Fig. 6), whereas a small population of them was also positive for CD20, CD79α, HLA-DR, IgM, HAM56, lysozyme (Fig. 7) and/or MAC387 (Table 2). Especially, CD3-positive small cells occupied 53% of the whole cellular population. Therefore, these small mononuclear cells were considered to be predominantly T-cells, with a very small number of histiocytic cells and non-neoplastic B-cells.

By dl-IF, it was revealed that the neoplastic large lymphoid cells were predominantly IgG-positive, but occasionally IgA- or IgM-positive. In addition, a few of the large lymphoid cells were double-labeled, in a combination of IgG+IgA or IgG+IgM (Fig. 8).

EBER-1 transcripts could not be detected in any neoplastic large lymphoid or reactive small mononuclear

---

**Table 1. Antibodies used in the present study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse monoclonal antibodies against</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human B Cell, CD20, BLA36</td>
<td>BioGenex</td>
<td>1:1000</td>
</tr>
<tr>
<td>Human B Cell, CD79α, HM57</td>
<td>DAKO</td>
<td>1:25</td>
</tr>
<tr>
<td>Human HLA-DR, Alpha-Chain</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>Human Macrophage, HAM 56</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>Human Myeloid/Histiocyte Antigen, MAC387</td>
<td>DAKO</td>
<td>1:100</td>
</tr>
<tr>
<td>Swine Vimentin, V9</td>
<td>DAKO</td>
<td>1:50</td>
</tr>
<tr>
<td>Human IgG</td>
<td>ZYMED</td>
<td>1:500</td>
</tr>
<tr>
<td>Rabbit polyclonal antisera against</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human T Cell, CD3</td>
<td>DAKO</td>
<td>1:300</td>
</tr>
<tr>
<td>Human IgG (Gamma-Chains)</td>
<td>DAKO</td>
<td>1:500</td>
</tr>
<tr>
<td>Human IgA (Alpha-Chains)</td>
<td>DAKO</td>
<td>1:300</td>
</tr>
<tr>
<td>Human IgM (Mu-Chains)</td>
<td>DAKO</td>
<td>1:300</td>
</tr>
<tr>
<td>Human lysozyme</td>
<td>DAKO</td>
<td>1:300</td>
</tr>
<tr>
<td>Human C3c Complement</td>
<td>DAKO</td>
<td>1:100</td>
</tr>
</tbody>
</table>

---

**Table 2. Immunohistochemical characteristics of the present lymphoma**

<table>
<thead>
<tr>
<th></th>
<th>CD20</th>
<th>CD79α</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>HLA-DR</th>
<th>CD3</th>
<th>HAM56</th>
<th>MAC387</th>
<th>lysozyme</th>
<th>vimentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastic large cell</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Small cell</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig. 1. Histology of the buccal mass in the cynomolgus monkey. Neoplastic large cells are intermingled with reactive small cells. Bar=40 μm. HE.

Fig. 2. Higher magnification of Fig. 1. In addition to neoplastic large cells and reactive small cells, a multinucleated giant cell (arrow) is seen. Bar=10 μm. HE.

Fig. 3. Immunostain for CD20. The majority of large cells show positive reaction. Bar=10 μm. Methylgreen counterstain.

Fig. 4. Immunostain for IgG. Most large cells show positive reaction. Bar=10 μm. Methylgreen counterstain.

Fig. 5. Immunostain for HLA-DR. The majority of large cells and small cells show positive reaction. Bar=10 μm. Methylgreen counterstain.

Fig. 6. Immunostain for CD3. The majority of small cells show positive reaction. Bar=10 μm. Methylgreen counterstain.

Fig. 7. Immunostain for lysozyme. Some small cells show positive reaction. Bar=10 μm. Methylgreen counterstain.

Fig. 8. Double-labeled immunoflorescence method. Neoplastic large cells are positive for IgA (green) or IgG (red), or both (yellow). Bar=5 μm.
cells in the buccal mass and mandibular lymph node.

Lymphomas showing a wide range of diverse morphology are well known in various mammalian species [18]. Furthermore, they have been immunohistochemically subclassified using lymphocytic subset markers [10]. In humans, TCRBCL is characterized by an admixture of large-sized neoplastic B-cells and small-sized reactive T-cells and the latter comprises more than 50% of the whole cellular population [19]. This type of lymphoma has been reported not only in humans [3, 19–21], but also in other mammalian species including dog [2], cat [6, 23], pig [26], and horse [17]. Histological features of TCRBCL in these animals are considered to be very similar to those of human TCRBCL.

In the present case, neoplastic large lymphoid cells, giant cells and reactive small mononuclear cells were observed. Neoplastic large lymphoid cells and giant cells showed B-cell markers, such as CD20, CD79α, Igs and HLA-DR, and the majority of the small mononuclear cells were CD3-positive and partly HLA-DR-positive reactive T-cells. These characteristics are consistent with those of TCRBCL in humans [3, 19–21]. However, in the present case some of the small-sized cells were positive for B-cell markers (CD20, CD79α) or histiocytic markers (HAM56, MAC387). Such a variety of reactive cells has not been confirmed in TCRBCLs reported in humans and other mammalian species [2, 3, 6, 17, 20, 21, 23, 26]. The difference between human and cynomolgus monkey may be due to different cytokine profiles produced by neoplastic B-cells.

Most B-cell lymphomas produce only a subclass of Ig, since all tumor cells usually originate from a single cell. The monoclonality of large B-cells in human TCRBCL has been reported in most cases, whereas polyclonality in TCRBCL has been demonstrated only in a pig [26]. The present study demonstrated polyclonal Ig production in B-cell lymphoma of a non-human primate using immunostains and the dl-IF method. These findings suggest that the neoplastic B-cells in this tumor may have produced polyclonal Ig. However, whether the neoplastic B-cells expressed Ig-κ and -λ remained to be confirmed. A relationship between EBV and oncogenesis was not demonstrated in the present case.

Acknowledgments

The authors thank Rieko Kobayashi for her technical help.

References