Abstract: Chediak-Higashi syndrome (CHS) is an autosomal recessive disorder characterized by severe immunologic defects including reduced bacteriocidal activity of neutrophils and impaired natural killer (NK) activity. The diagnostic feature of this syndrome is the presence of giant granules within cells. It was previously demonstrated that the abnormal down-regulation of protein kinase C (PKC) activity in cells from CHS patients and CHS (beige) mice is responsible for the cellular dysfunctions. The rapid down-regulation of PKC activity is found to be associated with the enhancement of calpain-mediated proteolysis of PKC. Moreover, it was reported that the levels in ceramide which is produced by sphingomyelinase (SMase) activation increased in CHS cells and the increased ceramide promotes the proteolysis of PKC. Meanwhile, two independent studies simultaneously identified the homologous genes that are mutated in human CHS and beige mice. The gene, named CHS1, encodes a protein of 3801 amino acids. CHS1 is a novel protein and was suggested to be important for lysosomal trafficking. However, the precise role of CHS1 has not been elucidated. To study the role of CHS1 protein in the regulation of PKC activity is of great importance to clarify the pathogenesis of CHS.

Key words: Chediak-Higashi syndrome, protein kinase C, CHS1 gene, beige mice, ceramide
as impaired natural killer (NK) activity, increased concanavalin A (Con A) cap formation and the giant granule formation. However, the relation between the CHS I and the regulation of PKC activity remains unknown. This review summarizes current findings on the pathogenesis of CHS.

**IMMUNOLOGIC DEFECTS IN CHS**

Patients with CHS have recurrent pyogenic infections such as *Staphylococcus aureus* and *Streptococcus pyogenes*. The most common infections occur in skin and the respiratory tract. Although the phagocytic ability of polymorphonuclear leukocytes (PMN) from CHS is normal, there is a delay in fusion of phagosomes with lysosomes. This delay permits bacterial replication. In addition, the activity of two lysosomal enzymes, elastase and cathepsin G, was selectively decreased in CHS and beige mice. These two enzymes were suggested to undergo similar processing in the Golgi apparatus, however, the reason why these two enzymes were defected remains to be clarified. On the other hand, Takeuchi et al. demonstrated that the inhibitor of these two lysosomal enzymes existed in extracts of beige neutrophils. However, the existence of the inhibitor has not been independently confirmed. In cells from CHS and beige mice, the proportion of Con A-capped cells increased compared with normal cells. The increment of Con A capping in CHS was suggested to be due to the defects in microtubule function or the abnormality in membrane fluidity.

The NK activity in CHS and beige mice was reported to be very low, although the number of NK cells is normal. Decreased NK activity was reported to be recovered by longer incubation with target tumor cells. It appears that the release of the cytolytic molecules such as perforin is delayed in NK cells from CHS. The function of cytotoxic T lymphocytes was also reported to be defective. In contrast, the production of antibody by B cells was not altered.

**ABNORMAL DOWN-REGULATION OF PKC IS ASSOCIATED WITH THE CELLULAR DYSFUNCTION IN CHS**

Several hypotheses concerning the pathogenesis of CHS have been reported. One of them is the defect in function of cytoskeleton. As mentioned above, Con A cap formation is increased in CHS cells. The cap formation was suggested to be associated with membrane-cytoskeleton interaction. Colchicine which disrupts microtubules increased the capping which resembles to the observation in CHS. So the defect of microtubule function is crucial in CHS. Another speculation on the pathogenesis of CHS is the increment of cyclic AMP levels. Carbachol which increases cyclic GMP levels was demonstrated to recover the abnormal capping in CHS. However, another study reported that the cyclic nucleotide levels were not altered in CHS. So it remains unclear whether the defect in cyclic nucleotide generation underlies this syndrome.

The PKC is a Ca\(_2\)+-phospholipid-dependent serine/threonine protein kinase and plays an essential role in intracellular signal transduction for various cell functions. At least 11 isoforms of PKC have been identified by investigators, that is, classical PKCs (\(\alpha\), \(\beta\)I, \(\beta\)II and \(\gamma\)), which are Ca\(_2\)+-dependent and activated by both phosphatidylinositol and diacylglycerol; novel PKCs (\(\delta\), \(\epsilon\), \(\eta\) and \(\theta\)), which are Ca\(_2\)+-independent and regulated by diacylglycerol and phosphatidylserine; and atypical PKCs (\(\zeta\), \(\iota\) and \(\lambda\)), which are Ca\(_2\)+-independent and do not require diacylglycerol for activation although phosphatidylserine...
regulates activity. The PKC isoforms are widely distributed in mammalian tissues. The roles of these PKCs are different among various cell types. Phorbol esters such as phorbol 12-myristate 13-acetate (PMA) activate PKC and cause a translocation from the cytosolic to the membrane fraction. After the translocation, the membrane-bound PKC is proteolyzed by calpain, which is a Ca²⁺-dependent thiol proteinase, to an inactive form. We previously reported that the membrane-bound PKC activity was rapidly down-regulated in NK cells, PMN, and fibroblasts from beige mice, and EB virus-transformed cell lines from CHS patients (Table 1). This breakdown of PKC was eliminated by pretreatment of cells with inhibitors of calpain, which protects PKC from its proteolysis. The calpain inhibitors such as (L-3-trans-carboxyoxirane-2-carbonyl)-L-leucyl-agmatine (E-64) and leupeptin corrected the impaired NK activity and increased Con A cap formation in beige mice. The improvement of cellular defects by various calpain inhibitors previously reported are shown in Table 2. E-64-d is a membrane-permeable analog of E-64 and it was recently reported that E-64-d corrected the giant granule formation in beige fibroblasts, and Con A cap formation in CHS cell lines.

### Table 1. Abnormal down-regulation of PKC in various cells from beige mice and CHS patients

<table>
<thead>
<tr>
<th>Cells</th>
<th>Stimulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beige mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells</td>
<td>Phorbol ester</td>
<td>13</td>
</tr>
<tr>
<td>PMN</td>
<td>Phorbol ester</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Con A</td>
<td>14</td>
</tr>
<tr>
<td>ChS patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Phorbol ester</td>
<td>15</td>
</tr>
<tr>
<td>Periphereral blood mononuclear cells</td>
<td>Con A</td>
<td>12</td>
</tr>
<tr>
<td>B cell lines</td>
<td>Con A</td>
<td>12</td>
</tr>
</tbody>
</table>

The membrane-bound PKC activity in beige mice and CHS patients was rapidly down-regulated after stimulation with phorbol ester or Con A. Details are described in the reference.

### Table 2. Improvement of cellular dysfunctions in beige mice and CHS patients by calpain inhibitors

<table>
<thead>
<tr>
<th>Cells</th>
<th>Imparement</th>
<th>Inhibitors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beige mice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK cells</td>
<td>NK activity</td>
<td>E64</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leupeptin</td>
<td>13</td>
</tr>
<tr>
<td>PMN</td>
<td>Con A cap</td>
<td>E64</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leupeptin</td>
<td>14</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Giant granule</td>
<td>E64d</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Elastase</td>
<td>E64d</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Cathepsin G</td>
<td>E64d</td>
<td>15</td>
</tr>
<tr>
<td>ChS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood mononuclear cells</td>
<td>Con A cap</td>
<td>E64d</td>
<td>12</td>
</tr>
<tr>
<td>B cell lines</td>
<td>Con A cap</td>
<td>E64d</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leupeptin</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z-Leu-Leu-H</td>
<td>12</td>
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<tr>
<td></td>
<td>Elastase</td>
<td>E64d</td>
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</tr>
<tr>
<td></td>
<td>Cathepsin G</td>
<td>E64d</td>
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</table>

Various cellular functions in CHS are improved by pretreatment of cells with calpain inhibitors. Details are described in the reference.
CHS cells. In contrast, PKC inhibitors such as chelerythrin, staurosporin and 1-(5-isooquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7) were shown to suppress elastase and cathepsin G activity\textsuperscript{12,15}, and NK activity\textsuperscript{31}. These inhibitors also increase Con A capping and promote giant granule formation\textsuperscript{14,15}. These findings strongly suggest that the abnormally rapid down-regulation of PKC is responsible for the cellular dysfunctions in CHS patients and beige mice.

**ALTERATION OF SPHINGOLIPID LEVELS IN CHS**

In an early study, sphingolipid turnover was suggested to be accelerated in leukocytes from CHS patients\textsuperscript{32}. Other studies reported that the composition of lipid in erythrocyte membrane in CHS is altered\textsuperscript{33}. These findings suggest that the lipid or sphingolipid levels may be altered in CHS cells. Meanwhile, a novel signaling pathway, termed the SM cycle, was identified by Okazaki et al.\textsuperscript{34}. SM is a major sphingolipid component of the mammalian cell membranes and is cleaved by a phospholipase C-like enzyme, sphingomyelinase (SMase), to generate the second messenger, ceramide. It is known that ceramide plays an important role in cell differentiation, cell proliferation, and or the induction of apoptosis\textsuperscript{34,55}. It was reported that ceramide production in beige mouse fibroblasts and CHS cell lines was enhanced by stimulation with Con A or PMA\textsuperscript{12,15}. Ceramide was reported to activate ceramide-dependent protein kinase\textsuperscript{36}, mitogen-activated protein kinase\textsuperscript{37} and protein phosphatase\textsuperscript{38}. Concerning its effect on PKC, it was reported that ceramide inhibits a translocation of PKC \(\alpha\) in murine epidermal and human skin fibroblasts\textsuperscript{39}, or inactivates PKC \(\alpha\) by activating protein phosphatase in Molt-4 cells\textsuperscript{38}. It was also reported that ceramide promotes calpain-mediated proteolysis of PKC \(\beta\) in murine PMNs\textsuperscript{40}. In addition, ceramide stimulates autophosphorylation of PKC \(\beta\). The PMA-induced down-regulation of PKC \(\beta\) was reported to require its autophosphorylation\textsuperscript{41}. Other studies have demonstrated that the autophosphorylation of residues in the hinge region, close to the site of cleavage by calpain, may alter the susceptibility of PKC \(\beta\) to proteolysis\textsuperscript{42}. So, the increased ceramide promotes calpain-mediated PKC breakdown by stimulation of autophosphorylation in CHS. The SMases include, at least, the acid lysosomal sphingomyelinase (A-SMase) and the neutral, membrane-associated Mg\textsuperscript{2+}-dependent SMase (N-SMase)\textsuperscript{43}. In a previous study, both A-SMase and N-SMase in CHS cells were activated after Con A stimulation\textsuperscript{12}. It is not clear which SMase is responsible for PKC breakdown. On the other hand, we also showed that cell-permeable ceramide analog (C\(_2\)-ceramide) induces PKC down-regulation in Con A- or PMA-stimulated cells. In addition, C\(_2\)-ceramide suppresses the elastase and cathepsin G activity and increases Con A capping in normal cell lines\textsuperscript{12}. In murine fibroblasts, C\(_2\)-ceramide was shown to promote giant granule formation\textsuperscript{15}. Taken together, the increased ceramide levels may be associated with the pathogenesis of CHS.

**THE GENETIC DEFECT OF CHS**

It was reported that the beige gene had been located to chromosome 13\textsuperscript{44} and the CHS gene to chromosome 1q42-q43\textsuperscript{45}. In 1996, two independent studies simultaneously identified the homologous genes that are mutated in human CHS and beige mice\textsuperscript{6,7}. The gene, named \textit{CHS 1}, encodes a protein of 3801 amino acids with a molecular weight of 430 kDa. Barbosa et al. reported that \textit{CHS 1} gene is disrupted by 5-
kilobase deletion and CHS 1 mRNA is markedly reduced in beige mice. In CHS patients, heterogeneous mutations of CHS 1 was reported. Karim et al. reported that missense mutant alleles that encode CHS 1 polypeptides with partial function were found in the adolescent and adult form of CHS. They also suggested an allelic genotype-phenotype relation among the various clinical forms of CHS. It therefore appears that CHS 1 has multiple functions. CHS 1 is cytosolic protein with three to four defined domains and was suggested important in MHC class II antigen presentation, or in the regulation of lysosomal fission. This protein has a region of sequence similar to stathmin, which associates with cellular signal transduction. Stathmin is a ubiquitous cytosolic protein which is phosphorylated at up to four sites in response to many signals and has many phosphorylation sites for PKC. However, the relation between CHS 1 and the phosphorylation by PKC is unknown. Another functional domain is called BEACH, which is homologous to Vps15, yeast vesicular sorting protein. Database searches have identified a family of eukaryotic proteins that contain BEACH domain. However, the precise role of CHS 1 protein has not been elucidated. The analysis of CHS 1 is of great importance to elucidate the pathogenesis of CHS and intracellular signal transduction.

TREATMENT FOR CHS

Since the bacteriocidal activity of neutrophils and NK activity are defective in CHS, it is important to protect patients from severe bacterial infections. The current treatment for infection is the use of antibiotics. However, this is only a temporary solution. The most effective treatment for CHS is bone marrow transplantation, which alleviates the immunologic problems, the most life-threatening defects. Some patients were treated by bone marrow transplantation, and the NK activity in those patients was reported to be improved. It was reported that ascorbic acid improved accelerated phase of CHS. In addition, predonine, vincristine and cyclophosphamide were suggested to be effective for the accelerated phase of CHS. However, these drugs are not effective for all CHS patients. More effective treatment including gene therapy is necessary to protect CHS patients from fatal problems.

CONCLUSIONS

Most of the characteristic abnormalities in CHS may be improved by elimination of the down-regulated PKC activity. The SMase activation induces ceramide production, and the increased ceramide enhances calpain-mediated proteolysis of PKC in CHS. Although the dysregulation of PKC is tightly associated with the pathogenesis of CHS, the relation between the regulation of PKC and CHS 1 has not been elucidated. Further studies on the role of CHS 1 and on the mechanism of PKC down-regulation will be necessary to clarify the pathogenesis of CHS and to find more effective treatment for CHS.

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