Clastic Cells of Hassall's Corpuscles During Acute Involution of the Thymus Induced by Cyclophosphamide in Guinea Pigs

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Summary. General and histochemical observations of the thymus were carried out in guinea pigs after injection of cyclophosphamide (280 mg/kg). Acute involution of the thymus induced by cyclophosphamide was accompanied by marked enlargement of Hassall's corpuscles in the first week after injection. However, the markedly enlarged Hassall's corpuscles disappeared entirely by the fourth week. Large cells characterized by pale nuclei with one or two prominent nucleoli became aggregated in the enlarged Hassall's corpuscles by the second week. Their cytoplasm frequently was foamy or vesicular in appearance. Histochemical observations revealed strong activities of nonspecific esterase, acid phosphatase and β-glucuronidase in these cells. Staining for these lysosomal hydrolytic enzymes was evident not only intracellularly but also extracellularly, indicating the dissolution of Hassall's corpuscles by intensive extracellular enzyme release.

The disappearance of enlarged Hassall's corpuscles accompanied by acute involution of the thymus after irradiation has been reported in cats (REGAUD and CREMIEU, 1911a, b), mice (DEANESLY, 1929) and guinea pigs (BLAU, 1967). However, the mechanism of the destruction of Hassall's corpuscles in such a short time is still unclear. Recently, the dissolution of Hassall's corpuscles by clastic cells, which show activities of acid phosphatase, β-glucuronidase and nonspecific esterase, has been reported in cyclic changes of Hassall's corpuscles, under physiological conditions, throughout the life span of sheep (KOTANI, FUKUMOTO and BRANDON, 1981), guinea pigs (KOTANI and NAWA, 1982) and humans (KOTANI, 1981). It is thus conceived that such clastic cells might play a major role in the destruction of Hassall's corpuscles during acute involution of the thymus. In the present study, the appearance of clastic cells in the Hassall's corpuscles undergoing destruction during acute involution of the thymus induced by cyclophosphamide was examined in guinea pigs.

MATERIALS AND METHODS

Animals and treatment with cyclophosphamide

Male Hartley guinea pigs, 5 weeks old and weighing 305-380 g, were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan), and fed with standard laboratory chow and water ad libitum under conventional conditions. A total of 15 animals were used in each of the experimental and control groups. Cyclophosphamide (Shionogi Pharmaceutical Co., Ltd., Osaka) was dissolved in physiological saline to a concentration of 25 mg/ml, and 280 mg/kg was injected intraperitoneally into the experimental animals. The control animals received saline only in the same manner.

Histology

Three animals from each of the experimental and control groups were sacrificed by ether inhalation at 0, 1, 2, 4, 6 weeks after intraperitoneal injection. After the thymus had been weighed, the right lobe was fixed in 10% formalin-saline, embedded in paraffin wax, cut serially at a thickness of 6 μm and stained with hematoxylin and eosin for general histologic observations. The left lobe was frozen in physiological saline to a concentration of 25 mg/ml, and 280 mg/kg was injected intraperitoneally into the experimental animals. The control animals received saline only in the same manner.

Sections air-dried at room temperature were fixed in
formol-calcium fixative (OGAWA, SHINONAGA and SAITO, 1962) at pH 7.2 for 3 min at 4°C, washed three times with distilled water and then incubated in the standard medium of BARKA and ANDERSON (1962) for acid phosphatase (substrate: α-methyl phosphate, pH 6.5), that of HAYASHI, NAKAJIMA and FISHMAN (1963) for β-glucuronidase (substrate: naphthol AS-BI glucuronide, pH 5.2), and that of YAM, LI and CROSBY (1971) for nonspecific esterase (substrate: α-naphthyl acetate, pH 6.4). Hexazotized pararosaniline was employed as a coupler for staining all these enzymes. Incubations for the staining of acid phosphatase, β-glucuronidase and nonspecific esterase lasted for 30 min at 37°C, 1 h at 37°C and 15 min at room temperature, respectively. After incubation, the sections were rinsed with distilled water and lightly counterstained with hematoxylin for 1-2 min.

RESULTS

Changes in thymus weight

The weight of the thymus decreased rapidly to about 30% of its normal value within 1 week after intraperitoneal injection of 280 mg/kg cyclophosphamide (Fig. 1). The low thymic weight persisted for a further week, and then the weight increased rapidly during the next 2 weeks until it slightly exceeded that of the controls. This rebound weight of the thymus returned to normal levels at 6 weeks after the cyclophosphamide administration.

General histologic observations of the thymus

In the first week after cyclophosphamide administration, the thickness of the thymic cortex was greatly reduced in comparison with that of the controls (Figs. 2, 3). However, apparently healthy lymphocytes were dispersed throughout the thin cortex, and no nuclear debris or fragments were evident (NETA et al., 1977). These findings suggested that the cortex was undergoing regeneration. The medulla was also markedly reduced in thickness and depleted of lymphocytes, although the involution was far less marked than that of the cortex. In parallel with the recovery of thymus weight, the thickness and cellularity of the thymus, particularly in cortex, increased rapidly in the fourth week and returned to normal histologic appearance by the sixth week.

In the medulla in the first week, Hassall’s corpuscles were conspicuous by increased both in number and size (Fig. 3). These enlarged Hassall’s corpuscles were mostly irregular-shaped cysts and contained various numbers of concentric corpuscles, suggesting that the former were enlarged by engulfing the latter. A distinct change occurred in Hassall’s corpuscles between the second and fourth weeks. Huge Hassall’s corpuscles disappeared entirely during this period, and many small ones reappeared in the medulla by the fourth week (Fig. 4). In the second week, large cells characterized by pale nuclei with a one or two prominent nucleoli were aggregated in the enlarged Hassall’s corpuscles (Fig. 5). Their cytoplasm frequently had a foamy or vesicular appearance. Occasionally, enlarged Hassell’s corpuscles were occupied by these cells.

Histochemical observations of the thymus

In the control thymus, cells strongly positive for nonspecific esterase were present preferentially in the...
Figs. 2-4. The thymus of a control guinea pig (Fig. 2) and of guinea pigs at 1 (Fig. 3) and 4 (Fig. 4) weeks after cyclophosphamide injection. C cortex, M medulla. Hematoxylin-eosin. ×140

Fig. 5. An enlarged Hassall’s corpuscle stained with hematoxylin-eosin at 2 weeks after cyclophosphamide injection. ×550
Figs. 6-9. Nonspecific esterase activity in the thymus of a control guinea pig (Fig. 6) and of guinea pigs at 1 (Fig. 7), 2 (Fig. 8) and 6 (Fig. 9) weeks after cyclophosphamide injection. C cortex, M medulla. ×140
Figs. 10-12. Enlarged Hassall's corpuscles stained for nonspecific esterase (Fig. 10), acid phosphatase (Fig. 11) and β-glucuronidase (Fig. 12) activities at 2 weeks after cyclophosphamide injection. ×550
de deepest cortex and in the medulla at the cortico-
medullary junction, although they were also scattered
diffusely throughout the cortex and medulla (Fig. 6).
The number of scattered cells was far lower in the
medulla than in the cortex.

During the first week after cyclophosphamide
administration, numerous nonspecific esterase-posi-
tive cells appeared in the shrunken cortex (Fig. 7).
Many of them were localized close to or in contact
with the walls of cortical blood vessels. Nonspecific
esterase-positive cells in the medulla appeared to
congregate around enlarged Hassall’s corpuscles,
frequently attaching to them. In the second week,
nonspecific esterase-positive cells in the cortex were
markedly decreased in number, and were found pre-
ferentially in the deepest cortex and in the medulla at
the corticomедullary junction (Fig. 8). In the medulla,
they became aggregated in and around the enlarged
Hassall’s corpuscles. The border of Hassall’s corpus-
cles invaded by these cells often became less distinct
in some or all parts. During the sixth week, the
distribution of nonspecific esterase-positive cells was
almost normal (Fig. 9). As well as being preferen-
tially localized in the deepest cortex and in the medulla
at the corticomедullary junction, they were frequent-
ly found along the blood vessels vertically penetrat-
ing the cortex. They were also occasionally attached
to slightly enlarged, newly formed corpuscles.

The time kinetics of the distribution of cells posi-
tive for acid phosphatase and β-glucuronidase were
quite similar to those of nonspecific esterase-positive
cells as described above. Enlarged Hassall’s corpus-
cles containing cells positive for nonspecific esterase,
acid phosphatase and β-glucuronidase at 2 weeks
after cyclophosphamide administration are shown in
Figs. 10, 11 and 12, respectively. The activities of
these lysosomal hydrolytic enzymes were stained not
only intracellularly but also extracellularly, indicat-
ing intensive extracellular release. Extracellular
release of these enzymes by cells located outside the
Hassall’s corpuscles was not evident.

DISCUSSION

The concentric Hassall’s corpuscles that increased in
number and size are enclosed in the lumen of cystic
Hassall’s corpuscles, which, histogenetically, have
the nature of a duct connecting the thymus to the
branchial pouch. However, they cannot be shed from
the thymus. The overgrown Hassall’s corpuscles are
destined for destruction within the thymus in order to
retain the lymphoid tissue within the organ. For this
purpose, the hydrolytic enzymes could conceivably
play a key role in the dissolution of corpuscles. Many
cells strongly positive for acid phosphatase, β-
glucuronidase and nonspecific esterase became ag-
ggregated within enlarged Hassall’s corpuscles by 2
weeks after cyclophosphamide administration, these
enlarged corpuscles then disappearing entirely by 4
weeks. Copious extracellular release of lysozomal
hydrolytic enzymes from these cells was noted in
corpuscles undergoing dissolution. An important
question then arises as to where such clastic cells of
Hassall’s corpuscles come together within the enlarg-
ed Hassall’s corpuscles. Active migration of macro-
phages from blood vessels at the corticomедullary
junction, which are rendered more permeable by
X-irradiation, in a direction inwards to Hassall’s
corpuscles has been reported by Blau (1967) during
acute involution of the thymus in guinea pigs. The
localization of the clastic cells at different times after
cyclophosphamide injection in the present study
strongly suggests that the clastic cells pass through
the blood vessels of the cortex, in which lymphoid
regeneration has just been initiated about 1 week
after injection, and then migrate from the cortex
through the medulla to aggregate within the Hassall’s
corpuscles by 2 weeks. The fate of clastic cells after
the destruction of Hassall’s corpuscles remains
nuclear. Only a few cells showing activities of acid
phosphatase, β-glucuronidase and nonspecific ester-
ase were scattered throughout the medulla at 4
weeks, when the huge Hassall’s corpuscles had
disappeared entirely. They may possibly be autolysed
in situ, scattered throughout the medulla or returned
to the circulation.

It was concluded that, during acute involution of
the thymus induced by cyclophosphamide, enlarged
Hassall’s corpuscles are completely destroyed in a
short time by clastic cells strongly positive for acid
phosphatase, β-glucuronidase and nonspecific ester-
ase, as is known to occur during cyclic changes in
Hassall’s corpuscles in the thymus of sheep (Kotani,
Fukumoto and Brandon, 1981), guinea pigs (Kotani
and Nawa, 1982) and humans (Kotani, 1981) under
normal physiological conditions throughout life.

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