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THE IMMUNOGLOBULIN NATURE OF RUSSELL BODIES

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Summary.—Although Russell bodies have been widely regarded as aggregates of immunoglobulin (Ig) their true nature remains controversial. This report reinvestigates the Ig reactivity of Russell bodies using an immunoperoxidase technique to detect IgG, IgA, IgM, kappa and lambda light chains and J chain on formalin fixed paraffin embedded tissue. Both intracellular and extracellular Russell bodies stained for Ig heavy and light chain determinants. Generally staining was most intense at the surface of these structures although many appeared to have detectable Ig determinants within the Russell body cores. The results suggest that Russell bodies, irrespective of size or site, are associated with intact Ig molecules.

RUSSELL BODIES are relatively common within plasma cells and follicle centre cells at sites associated with high antigen load (Weill, 1920; Lisco, 1942; White, 1954), within neoplastic lymphoid cells (Rappaport and Johnson, 1955; Brunning and Parkin, 1976; van den Tweel *et al.*, 1978) and extracellularly in such situations. The majority of histochemical, ultrastructural and immunofluorescence studies lend support to the idea that they are composed of aggregates of immunoglobulin light chains or intact immunoglobulin (Ig) (White, 1954; Goldenberg and Deane, 1960; Welsh, 1960; Fisher and Zawadzki, 1970; Blom, Mansa and Wilk, 1976). However, the nature of Russell bodies remains unresolved and recent immunoperoxidase studies have yielded contradictory results (van den Tweel *et al.*, 1978; Hsu *et al.*, 1982). This paper reinvestigates the Ig reactivity of Russell bodies using an immunoperoxidase technique on formalin fixed paraffin embedded tissues.

MATERIALS AND METHODS

Tissue from inflammatory lesions of the oral mucosa (4), gingiva (4) and palatine tonsil (4) containing Russell bodies were available in the Department of Oral Pathology, University of Birmingham as stored wax blocks. Russell bodies in all specimens stained pink with haematoxylin and eosin and were PAS positive. All tissues had been fixed in neutral buffered formalin (18–24 h) and processed to paraffin wax via ethanol and xylene. Five micron sections were mounted on clean, adhesive-free slides and dried at 56° for 45 min before immunostaining.

The unlabelled antibody peroxidase-antiperoxidase (PAP) complex method was performed, with prior trypsinization of sections, as described previously (Matthews and Basu, 1982*a*, *b*). The standard dilutions and times for each antiserum overlay were as follows: rabbit anti-human IgG*, IgA*, IgM*, kappa* and lambda* light chains (1:1000), anti-human J chain† (1:250) 60 min; swine anti-rabbit Ig* (1:50) 30 min; PAP complex* (1:50) 30 min. All sections were counterstained with Mayer's haematoxylin after visualisation of bound peroxidase using diaminobenzidene reagent. Controls included omission of the primary and secondary layers, replacement of the primary layer with normal rabbit serum and specific inhibition of positive staining by purified antigens.

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RESULTS

Intense staining for Ig was obtained in the cytoplasm of plasma cells within all sections. The majority contained IgG, some IgA and a few IgM with more kappa than lambda chain positive cells. Weak cytoplasmic staining for J chain was obtained for most plasma cells, however strong reactivity was limited to a small number of presumably IgM or dimeric IgA containing cells (Korsrud and Brandtzaeg, 1981). The histological diagnosis, types of Russell bodies present and their staining characteristics are summarized in the Table. All tissues contained intracellular Russell bodies, usually of small size (< 9 μm diameter) and within the cytoplasm of mature plasma cells. In one tonsil specimen, however, Russell bodies were only detected within the cytoplasm of follicle centre cells from a number of different germinal centres (Table; Fig. 1). Extracellular Russell bodies were less common and usually medium and/or large in size (10–14 or > 14 μm diameter; Fig. 2). All types of Russell bodies showed a similar pattern of staining for Ig with dark rim-like staining often equivalent to the intensity found in plasma cell cytoplasm and paler core staining (Figs 1 & 2). All tissues contained IgG positive Russell bodies some of which, with the exception of specimen 4, were positive for kappa and others lambda chains. Thus, when staining for Ig light chain types negative Russell bodies, although difficult to find, were usually present. Four specimens showed small numbers of IgM positive Russell bodies one of which also contained some stained for IgA (Table). Sections stained for IgM and IgA contained large numbers of negative Russell bodies. A single specimen showed a few intracellular J chain positive Russell bodies (specimen 3) which presumably corresponded to those positive for IgM. Specimen 4 was unusual in that both intra- and extracellular Russell bodies stained monoclonally for IgG and kappa chains even though plasma cells positive for all three Ig classes and both light chain types were detectable.

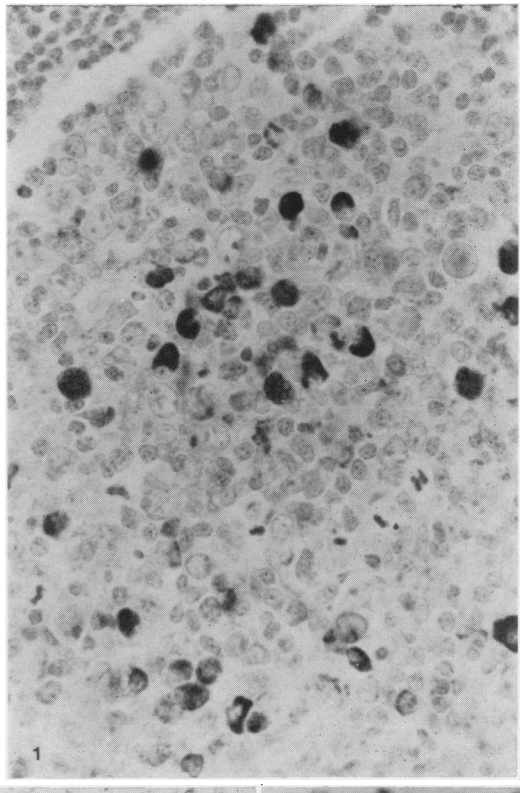
DISCUSSION

The results of this study suggest that Russell bodies of various sizes and present intra- and extracellularly at inflammatory sites are positive for Ig determinants. It is also apparent from the ability to stain these structures for either heavy or light chains that they are associated with intact, native Ig molecules rather than light chains produced in excess (Fisher and Zawadzki, 1970) or antigenically denatured Ig (Hsu *et al.*, 1982).This is in accord with the immunofluorescence results of Dillman and co-workers (Dillman *et al.*, 1982) who demonstrated monotypic core staining for Ig of intra- and extracellular globules resembling Russell bodies in the liver and bone marrow of a patient with monoclonal gammopathy. However, these globules were stained grey with haematoxylin and eosin and were PAS negative in contrast to the eosinophilic, PAS positive Russell bodies studied here. A recent immunoperoxidase study of non-Hodgkins lymphoma has also demonstrated both core and surface staining of eosinophilic, PAS positive Russell bodies for Ig heavy and light chains (van den Tweel *et al.*, 1978). This work indicated that Dutcher bodies as well as vacuoles and fibrillar inclusions were also positive for Ig and suggested that there was a developmental relationship between the different types of Ig inclusion.

The failure of Hsu and co-workers (Hsu *et al.*, 1982) to obtain Ig staining of Russell bodies at the light microscopic level is difficult to understand as they used an immunoperoxidase technique essentially similar to that used in the present study. These workers also studied a gut lymph-

FIG. 1.—Russell bodies within follicle centre cells of palatine tonsil (specimen 9). PAP method for IgG; Mayer's haematoxylin counterstain. $\times 400$.

FIG. 2. (a) Large, medium and small, intra- and extracellular Russell bodies in residual minor salivary gland tissue (specimen 4). PAP method for kappa chains; Mayer's haematoxylin counterstain. $\times 144$. (b) Higher power view of part of Fig. 2(a) $\times 360$.



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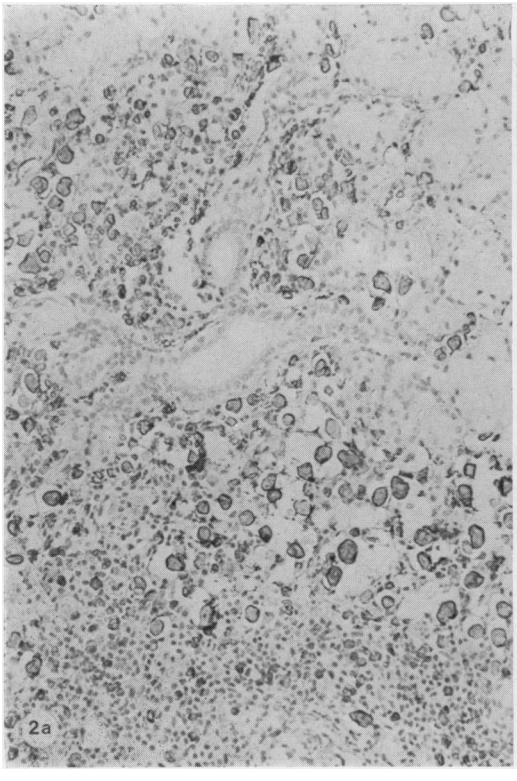

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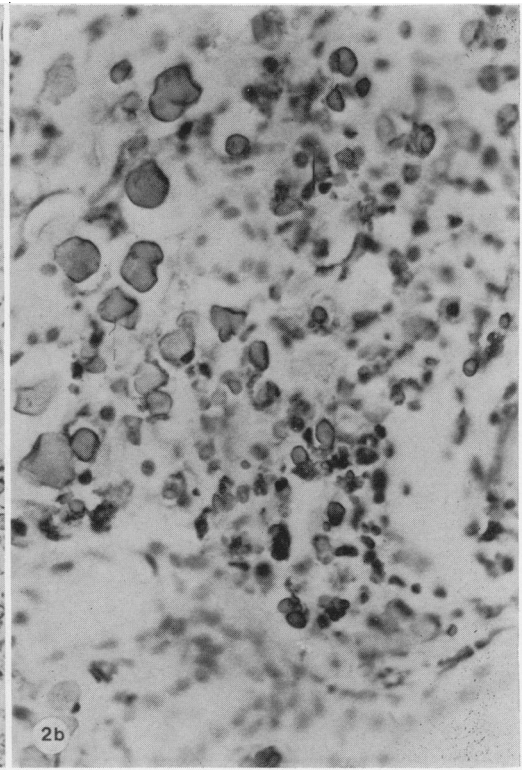

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TABLE.—*Summary of staining results*

			Site/size of Russell bodies*						Immunoperoxidase staining results					
			Intracellular			Extracellular			Intracellular			Extracellular		
No.	Site	Diagnosis	S	M	L	S	M	L	Rim	Core	Ig stain	Rim	Core	Ig stain
1	Upper lip	Chronic mucositis	+	+	(+)	−	+	(+)	+++	+	G κλ	+++	+	G κλ
2	Buccal sulcus	Reactive lymphoid tissue	+	−	−	−	−	−	++	+	G κλ		N/A	
3	Buccal mucosa	Non-specific inflammation	+	+	(+)	−	−	+	++	++	G(A, M) κλ (J)	++	+	G(M) κλ
4	Labial mucosa	Sjorgrens disease	+	+	+	(+)	+	+	+++	++	G κ	+++	++	G κ
5	Gingiva	Plasma cell gingivitis	+	+	−	−	−	−	+++	++	G κλ		N/A	
6	Gingiva	Chronic gingivitis	+	−	−	−	−	−	++	+	G κλ		N/A	
7	Gingiva	Chronic gingivitis	+	+	−	−	−	−	++	+	G(M) κλ		N/A	
8	Gingiva	Chronic periodontitis	+	−	−	(+)	(+)	−	++	++	G κλ	+	+	G κλ
9	Palatine tonsil	Reactive hyperplasia	+†	−	−	−	−	−	+++	+	G κλ		N/A	
10	Palatine tonsil	Reactive hyperplasia	+	−	−	−	−	−	++	+	G κλ		N/A	
11	Palatine tonsil	Reactive hyperplasia	+	+	−	−	(+)	−	++	+	G κλ	++	+	G κλ
12	Palatine tonsil	Reactive hyperplasia	+	−	−	−	(+)	(+)	++	++	G(M) κλ	+	+	G κλ

* S, M, L refer to small, medium and large Russell bodies.
† Russell bodies found in follicle centre cells only.
Brackets indicate an unusual positive finding.
N/A = not applicable.

oma possessing Russell bodies by immunoelectron microscopy and demonstrated apparent merging of Ig positive flocculant material with negative Russell body cores. This result may indicate that Russell bodies are not Ig in nature. However, the apparent lack of staining could have been the result of limited penetration of reagents and the possibility remains that Russell bodies are due to a condensation process of intercisternal Ig.

In conclusion the majority of available evidence supports the concept that Russell bodies are composed of, or are intimately associated with, Ig. Further work involving histochemical and immunohistochemical methods at both light and electron microscopic levels on the various types of Russell bodies may finally resolve the controversy over the nature of these structures.

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