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Ultrastructure of cells infiltrating human kidney allografts

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SUMMARY

Cell infiltration is commonly observed in human renal allograft biopsies. This infiltration was investigated using electron microscopy for a more precise assessment of the nature of these cells. More than 3000 cells infiltrating twenty-five renal allograft biopsies were studied. Six cellular types were distinguished and a mean percentage of each type was calculated. Only one-half of these cells were normal or transformed lymphocytes (including small lymphocytes: $22{\cdot}3 \pm 3{\cdot}8\%$, 'intermediary' cells: $22 \pm 3{\cdot}6\%$, blast-like cells similar to MLC transformed lymphocytes: $8{\cdot}1 \pm 2{\cdot}4\%$. A relatively high number of plasmocytes ($12{\cdot}4 \pm 2{\cdot}5\%$) and a still higher percentage of macrophages ($28{\cdot}5 \pm 4{\cdot}6\%$) were found. Granulocytes represented only $2 \pm 0{\cdot}8\%$ of the cell population. Variations of the mean percentage of these cellular types were studied in various clinical situations.

INTRODUCTION

Some cell infiltration is often found in human renal allograft biopsies. Interstitial cells appear numerous during rejection episodes but may also be found in the form of focal infiltrates in renal allografts showing perfectly satisfactory function.

Previous studies based on light microscopy describe these infiltrates as mainly composed of round basophil, mononuclear, lymphoid-like cells (Rosenan, Lee & Najarian, 1969; Rowland *et al.*, 1970; Hamburger *et al.*, 1965, 1971; Lindquist *et al.*, 1968; Porter, 1964; Porter *et al.*, 1966). Electron microscopy is necessary for a more precise and accurate description. Apart from the original papers of Galle & de Montera (1962) and of Porter (1965), little has been published about the ultrastructure of these cells. The present study is based on the ultrastructural description of more than 3000 cells infiltrating twenty-five human renal allograft biopsies.

MATERIALS AND METHODS

Renal biopsies were performed between 7 days and 4 years after transplantation, using a previously described procedure (Hamburger *et al.*, 1971). A total of twenty-five biopsy specimens were studied: thirteen cases during early (six cases) or late (seven cases) graft rejection episodes, and twelve cases with excellent graft function. The biopsy specimens were fixed in 3% glutaraldehyde with 0·1 M phosphate buffer, postfixed in 1% osmium tetroxide, dried, and embedded in Epon. Sections were 900 Å thick. They were stained with uranyl acetate and lead citrate. Nine blocks of each biopsy were submitted to examination. Various serial section micrographs were made of all cells invading the interstitium. Cell structure studies were based on approximately 300 micrographs per biopsy using the following criteria: general cell shape, nucleocytoplasmic ratio, appearance of the nucleus, chromatine arrangement, appearance of the nucleoles, distribution of RNA, and other cell components, i.e. mitochondria, Golgi apparatus, vacuoles and lysosomes.

RESULTS

The above-mentioned structural criteria led to a classification of the observed cells into six different types: three subgroups of lymphoid cells (Types I, II and III), plasma cells (Type IV), macrophages (Type V) and granulocytes (Type VI).

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Type I: small lymphocytes (Figs 1 and 2)

A percentage of 22·8±3·8 of all infiltrating cells were classified as small lymphocytes because of the following features: approximately 8 μm in diameter, a high nucleocytoplasmic ratio and a round, large and indented nucleus. The heterochromatin formed heavy clumps in the nucleoplasm and along the nuclear membrane. Depending upon the section, a nucleolus could sometimes be seen. The cytoplasm was clear and finely granular with few organelles, i.e. three or four mitochondria, few and dispersed

FIG. 1

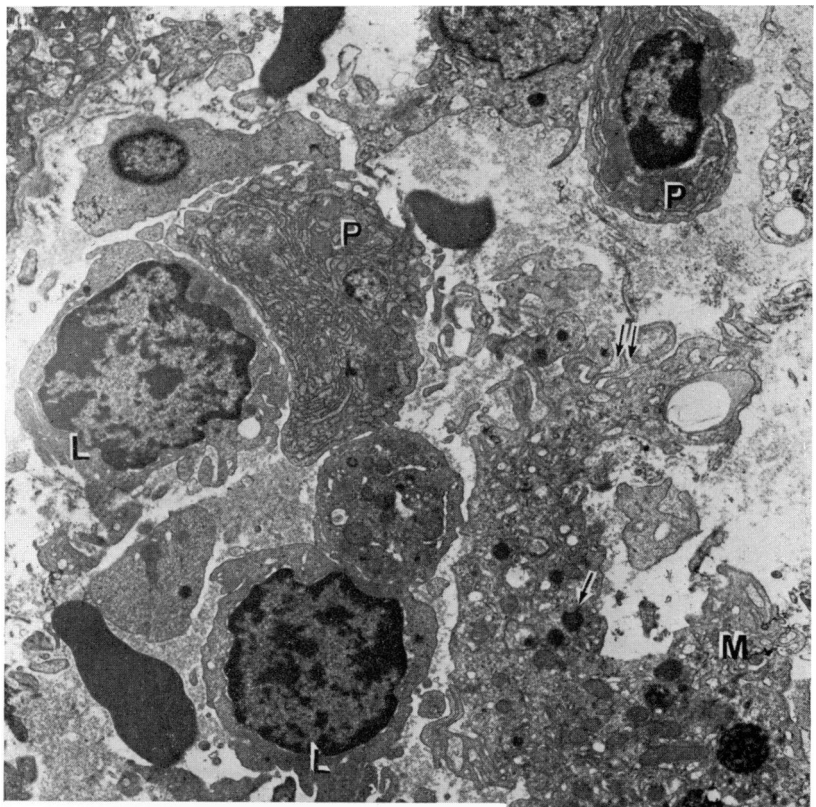

FIG. 2

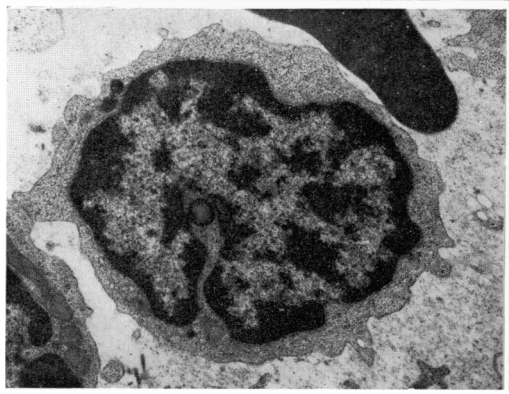

FIG. 1. General view showing three cellular types in the interstitium. We observe two typical small lymphocytes (L), two plasma cells (P) and a macrophage (M) with numerous pseudopodia (⇉), vacuoles and lysosomes (→) scattered through the cytoplasm. (Magnification × 3500.)

FIG. 2. Insert from the preceding micrograph showing cell Type I of our classification. It is a typical small lymphocyte with indented nucleus and high nucleous to plasmic ratio. (Magnification × 5600.)

ribosomes, occasional polyribosomes, one or two ergastoplasm profiles, one or two dense granules and a few Golgi apparatus.

Type II: intermediate cells (Fig. 3)

An average of 22·1±3·6% of the infiltrating cells were termed 'intermediate cells' because of their morphological similarity with cells observed during the early stages of blast transformation in mixed lymphocyte cultures (Bain, Vas & Lowenstein, 1964; Tanaka *et al.*, 1963). These cells were approxi-

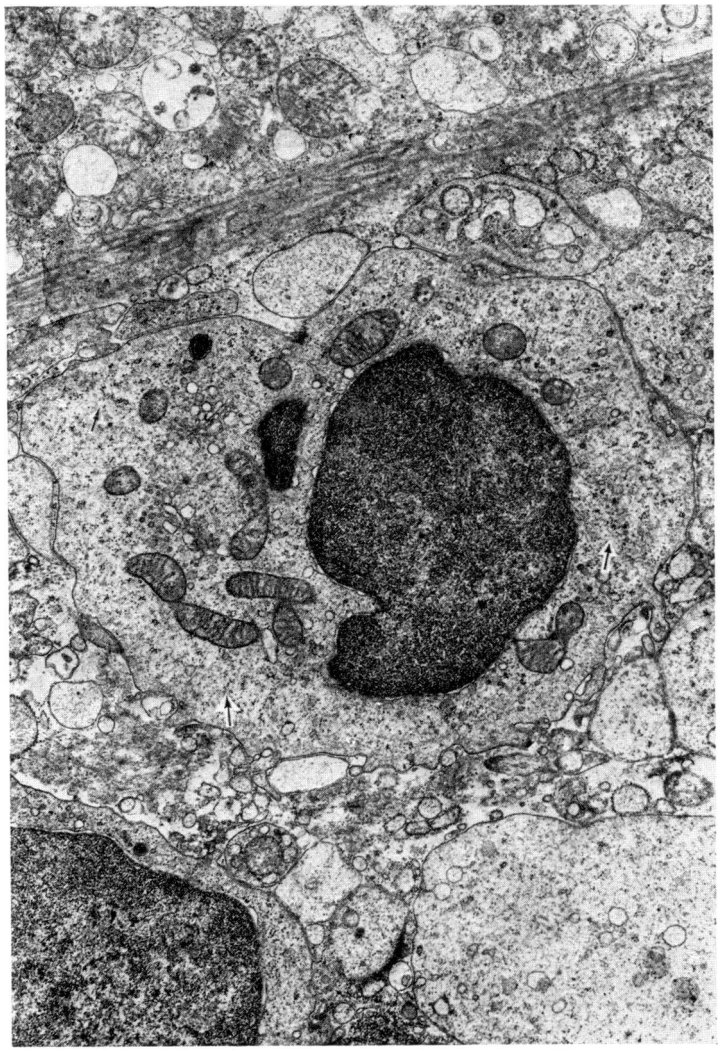

FIG. 3. Type II of our classification. This cell was termed 'intermediate' because of its morphological similarity with cells observed during the early stages of blast transformation in MLC. We can see the dispersed chromatin enclosed in the nucleus. On another section, a prominent nucleolus could be observed. Cytoplasm is large, containing free ribosomes (→), mitochondria and Golgi apparatus. (Magnification × 7500.)

mately 12 μm in diameter, with a regular nucleus containing thin and dispersed chromatin and one or two hypertrophied reticulated nucleoli. The cytoplasm contained single ribosomes and a few polyribosomes. Mitochondria were rare and the ergastoplasm as well as the Golgi apparatus were only slightly developed.

Type III: blast-like cells

Fig. 4 shows this large cell (approximately 15 μm in diameter) with a large and irregular nucleus containing thin chromatin spreading throughout the nucleoplasm and occasionally aggregating along the nuclear membrane, with one or two prominent nucleoli. The cytoplasm is abundant. It contains numerous free ribosomes forming 'rosettes' and several mitochondria. The Golgi apparatus is more developed than in Type II.

These blast-like lymphocytes represented $8{\cdot}1 \pm 2{\cdot}4\%$ of the infiltrating cells.

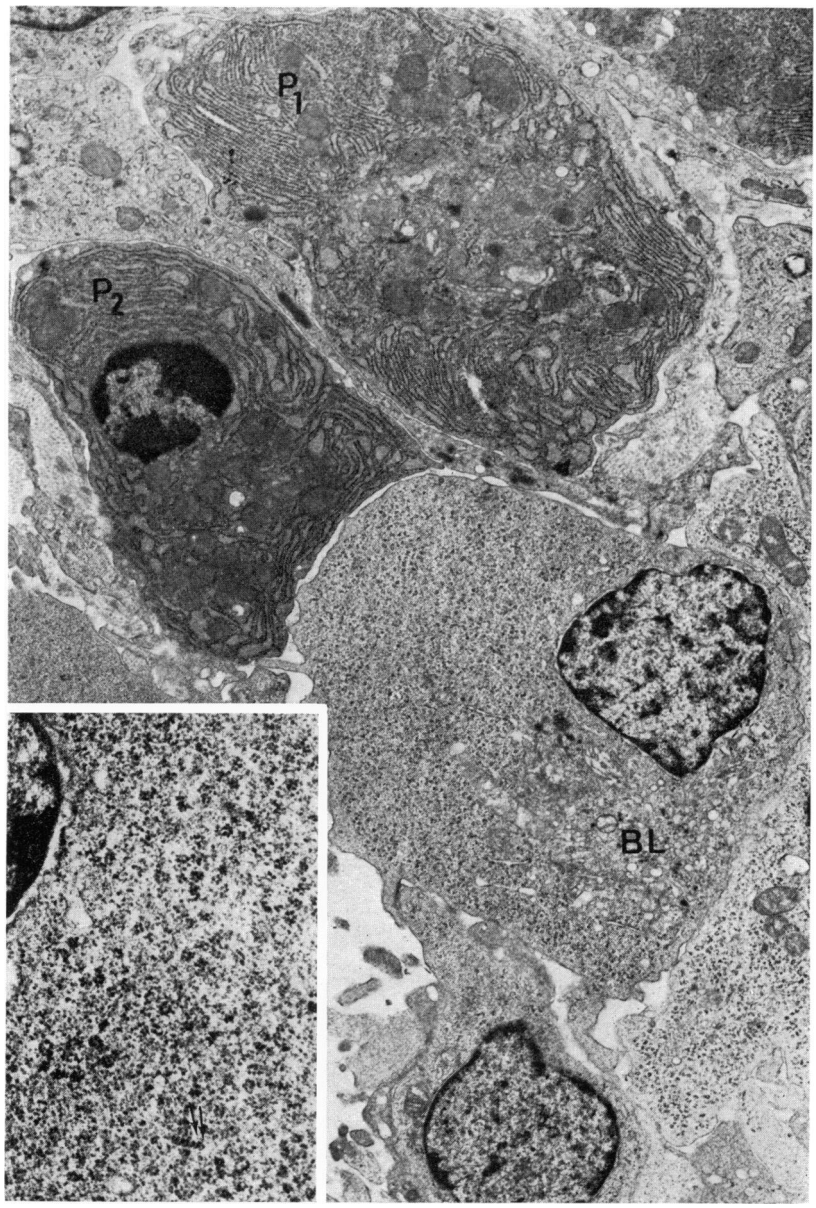

FIG. 4. In this micrograph we can observe Type III, a blast-like cell (BL) with numerous aggregated ribosomes in the well-developed cytoplasm. This section also shows cellular Type IV, typical plasma cells with ergastoplasmic lamellae invading the cytoplasm, except in the clear zone of the Golgi apparatus in the case of P_1. Another section of the same type of cell (P_2) shows the nucleus containing chromatin clumps. Insert: a high magnification of the blast-like cell cytoplasm containing aggregated ribosomes forming 'rosette' and chains ($\rightrightarrows$). (Magnification $\times$ 16,800.)

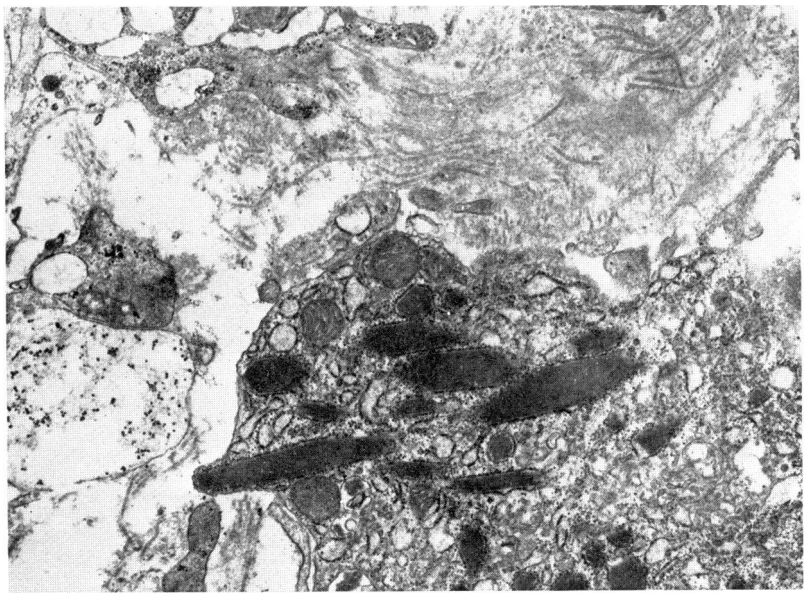

FIG. 5. Mature plasma cell containing some distended cisternae filled with crystal-like material. (Magnification × 12,000.)

Type IV: plasma cells (Figs 1 and 4)

Cells with the classical appearance of plasma cells represented 12·6±2·5% of all cells. They were large, ovoid elements (approximately 15 μm in diameter) with an oval, eccentric nucleus, containing six or eight compact chromatin clumps marginated in the nuclear periphery, with a medium-size nucleolus. Except in the perinuclear zone which remained clear and contained well-developed Golgi apparatus, the endoplasmic reticulum invaded practically the whole cytoplasm.

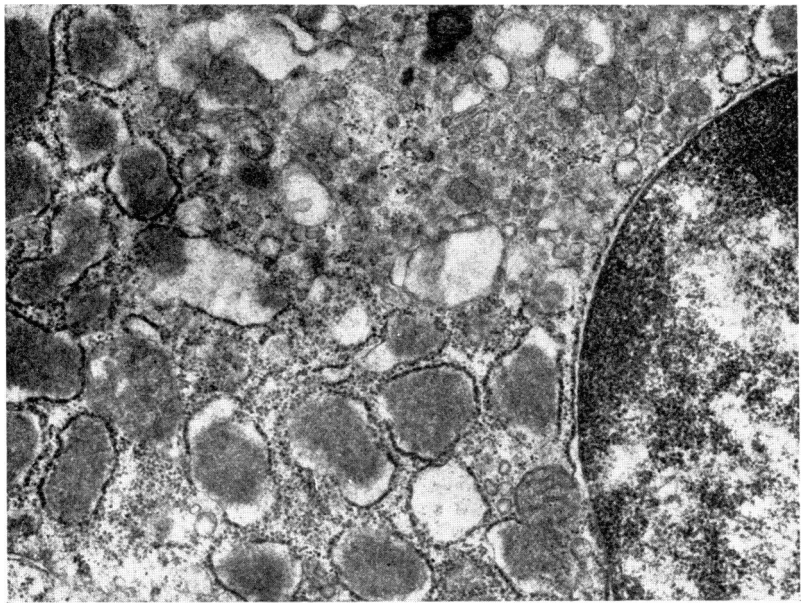

FIG. 6. High magnification of plasma cell containing distended cisternae filled with homogeneous dense material similar to Russel's bodies. (Magnification × 20,000.)

These ergastoplasmic saccules sometimes appeared dilated, containing either an homogeneous material moderately dense to electrons, or fine granules or flakes, sometimes crystal-like (Fig. 5). When numerous, these saccules were very similar to Russel bodies (Fig. 6).

In some cells, aggregated ribosomes and small chains or spirals of polysomes, together with numerous ergastoplasmic profiles and an eccentric nucleus with some chromatin clumps, suggested an intermediary

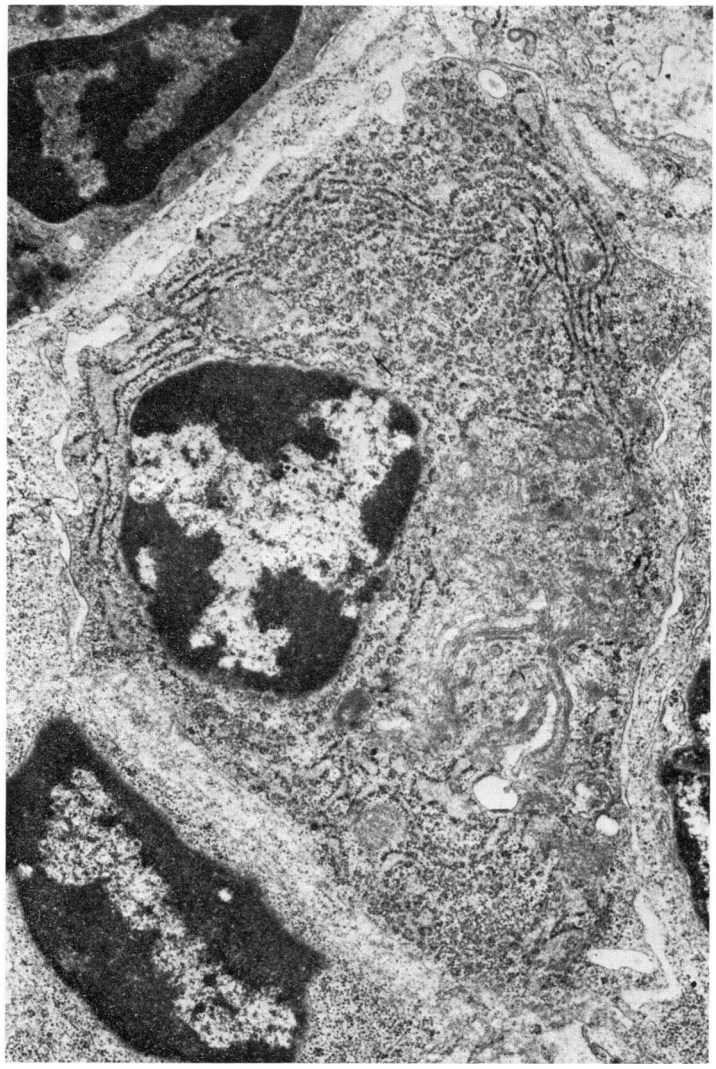

FIG. 7. This cellular type shows specific characteristics: aggregated ribosomes and small chains and spirals of polysomes together with ergastoplasmic lamellae, and an eccentric nucleus containing some chromatin clumps. They suggest an intermediate stage between Types III and IV. (Magnification × 8750.)

stage which will be discussed later (Fig. 7). Moreover, in order not to complicate our classification with too many subgroups, these cells, as well as other immature plasma cells, were included within Type IV.

Type V: macrophages (Figs 1 and 8)

A percentage as high as $28{\cdot}5 \pm 4{\cdot}6$ of all cells presented the appearance of macrophages with numerous digitations and many pseudopodia. The nucleus was irregular, containing dispersed chromatin and

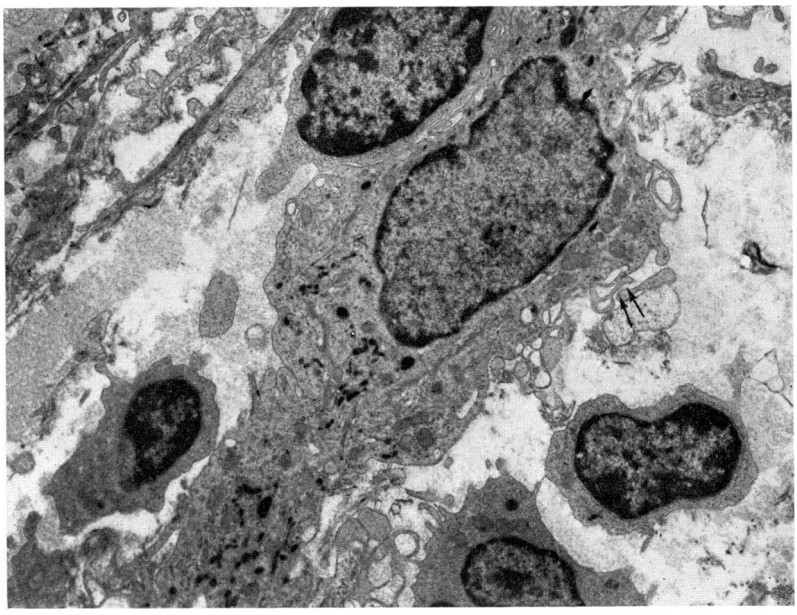

FIG. 8. Another view of a macrophage (Type V) showing digitations with phagocytosis and pinocytosis (⇉). The cytoplasm is rich in granulations. (Magnification × 4800.)

inconsistently a nucleolus. The cytoplasm was rich in organelles, multivesicular bodies, lysosomes and vacuoles of various electron density. Images of pinocytosis and phagocytosis were often seen.

Type VI: granulocytes

An average of 2·5 ± 0·8% of all cells were classical polymorphs, with neutrophil, eosinophil or basophil granules.

Table 1 summarizes the average percentage and standard deviation of the various types of cells described above.

TABLE 1. Percentages of various types of cells observed

Type I	Small lymphocytes	22·8 ± 3·83%
Type II	Intermediate cells	22·1 ± 3·6%
Type III	Blast cells	8·1 ± 2·1%
Type IV	Plasma cells	12·6 ± 2·5%
Type V	Macrophages	23·2 ± 4·6%
Type VI	Granulocytes	2·5 ± 0·8%

Clinical correlations

We further compared the percentage of various cells with graft function at the time of biopsy, as shown in Table 2.

No significant difference in the proportion of small lymphocytes (Type I), intermediate cells (Type II) or plasmocytes (Type IV) was found between biopsies performed during early rejection episodes (before the end of the first month post-transplantation), late rejection episodes (after the first month) or when the graft function was perfect.

Conversely, the percentage of transformed cells (Type III) was significantly higher (12·8%) during

TABLE 2. Percentages of infiltrating cells in various clinical situations

	Type I	Type II	Type III	Type IV	Type V	Type VI
Early rejection crises	18±7	19·6±7·5	17·2±5·6	12±5	26±5·9	5·7±4·2
Late rejection crises	23·2±5·2	22·8±3·5	8±2·9	10·8±1·9	33±3·2	0·8±1·2
Total rejection crises	20·6±1·3	21·2±3·95	12·6±3·4	11·3±2·4	29·3±3	3·2±3
Stable and satisfactory renal function	25±7·6	23·8±3	3·6±1·4	14±5·4	17·2±5·9	1·9±2·6

rejection episodes than in cases of normal function (3·6%). The difference was less striking in late (8%) than in early crisis (17·2%).

A slight difference in the percentage of macrophages (Type V) was found when comparing rejection periods (29·5%) and cases with good renal function (17·2%). The difference between these two figures is not, however, statistically significant.

The percentage of polymorphonuclear leucocytes was slightly higher during rejection episodes (3·2%) particularly during early crisis (5·7%) than in patients with a perfect and stable renal function (1·9%).

DISCUSSION

It is clear today that a purely morphological definition of cells is far from satisfactory for the identification of cells infiltrating allografts. In fact, no sound classification can be obtained with light microscopy. This is probably why previous descriptions have been rather vague concerning the percentage of each type of cell or even the exact nature of these cells. Despite evident limitation of a purely descriptive approach, electron microscopy is a much safer method for comparing the observed cells to the usually accepted nomenclature of white blood cells, even if this nomenclature may seem arbitrary or incomplete in view of recent advances in the knowledge of the natural history and function of these cells.

Cells infiltrating the experimental renal allograft have been more extensively studied than those in the human. Several works have been published on the rat (Feldman & Lee, 1967; Guttman *et al.*, 1967; Lindquist *et al.*, 1971); on the dog (Dempster & Williams, 1963; Porter *et al.*, 1964; and on the sheep (Pedersen & Morris, 1970). In the rat, Lindquist *et al.* (1971) described four main cell types: small lymphocytes, blast cells, plasma cells and macrophages; they also mention intermediary cells between the first three types. Blast cells and lymphocytes are mainly found before the 7th day post-transplantation, after which the majority of infiltrating cells is small lymphocytes. In the dog, Porter (1964) found the appearance of small lymphocytes in peritubular capillaries as soon as 24 hr after transplantation. At 48 hr, he found the appearance of pyroninophil cells, which he separates into two groups, the first representing blast-like cells with numerous ribosomes and the second corresponding to plasma cells. In the sheep (Pedersen & Morris, 1970), blast cells, plasma cells and some macrophages have been observed. We ourselves have observed in the experimental renal graft of the rat the six cell types described in the present paper, with similar percentages (Nabarra, 1976).

As far as we know, the present work is the first quantitative study in the human. In order to follow as closely as possible the present ultrastructural criteria defining the various cell types, we based our classification on descriptions found in the following references: Rebuck & Lo Grippo (1961); Binet & Mathé (1961); Hall *et al.* (1966); Harris *et al.* (1966); Mori & Lennert (1969); Bessis (1975). Six main cell types were distinguished: Type I (classical small lymphocytes), Type II (intermediary cells as seen in mixed lymphocytecultures), Type III (blast-like cells), Type IV (plasma cells), Type V (macrophages), Type VI (granulocytes). Our results are in good agreement with the preliminary non-quantitative study performed some years ago in this same laboratory by Galle & de Montera (1962).

Types I, II and III cells, representing a little more than 50% of the total of infiltrating elements, are quite similar to those observed in a mixed lymphocyte culture after the 4th or 5h day. Since the mixed lymphocyte culture has been considered as a simplified *in vitro* model of the allogenic immune

reaction, the finding of a similar series of cells within the grafted kidney could be interpreted as the *in vivo* equivalent of the lymphocyte stimulation and transformation described *in vitro*. Hamburger *et al.* (1971) have shown that such a transformation actually occurs in human renal grafts by studying the efferent lymph of the transplanted kidney day after day following transplantation. Using chromosomic markers when donor and recipient were of different sex, they showed that all blast-like transformed lymphocytes belonged to the recipient. Hence, it may well be that Types I, II and III of our description represent the sequence of the host lymphoid stimulation.

It is difficult to go further in the identification of the various lymphocyte subpopulations. Strom *et al.* (1975), studying cells extracted from allografted kidneys which had been removed and ground, have used various tests such as surface immunoglobulin detection, rosettes, sensitivity to complement and adher-

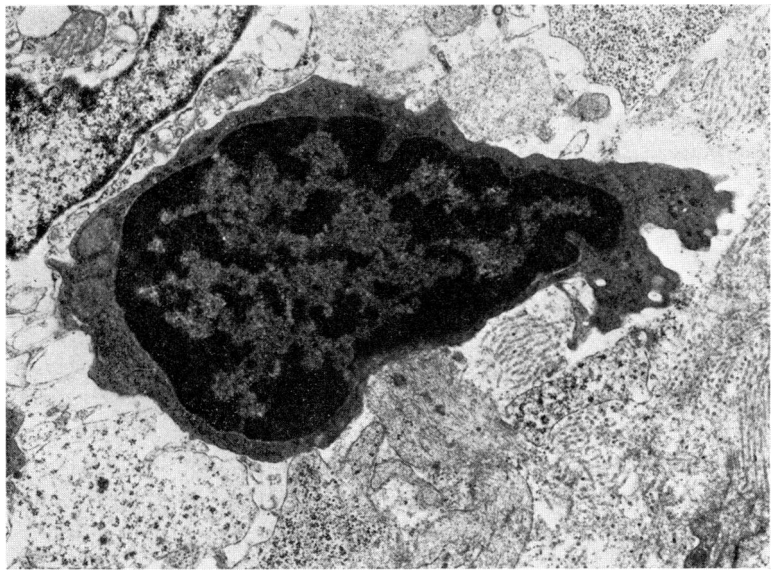

FIG. 9. Micrograph showing a peculiar small lymphocyte, with very dense cytoplasms and a uropod. (Magnifition × 6400.)

ence, in order to evaluate the respective proportion of B and T lymphocytes. They found 18–73% of B lymphocytes compared with 8–72% of T lymphocytes. Electron microscopy determination alone is still insufficient at present to recognize with certainty the various functional subpopulations of lymphocytes. In spite of the work of Matter *et al.* (1972), Polliak *et al.* (1973), Alexander & Wetzel (1975) and others, there is not yet a complete agreement in the description of the ultrastructural features specific to each lymphocyte subpopulation. The fact that we saw different chromatin and cytoplasmic patterns among the infiltrating lymphocytes could correspond to simultaneous presence of various lymphocytes, including T and B cells. A few cells observed by us showed an electron dense cytoplasm and an ovoid shape with an irregular uropod similar to that termed T-3 by Matter (Fig. 9). Among blast-like cells, however, we could not find the distinction between blast cells of T or B origin as described by that author.

The presence of B cells and their differentiation towards plasma cells was supported in our observations by the presence of intermediary cells showing ribosomes aggregated in rosettes, chains or spirals and ergastoplasmic profiles surrounded by a certain number of ribosomes.

The high number of macrophages (Type V) in our biopsies was striking and somewhat unexpected. This cell type is more often reported in experimental models than in human transplantation. However, Yamanaka *et al.* (1976) have underlined the large number of macrophages in early acute and severe rejection of human renal allografts. These observations can perhaps be compared with the increasing importance attached to the phenomenon of 'macrophage arming' among the effector mechanisms of allograft immunity (Dimitriu *et al.*, 1975).

The study of possible correlations between the respective percentages of various cell types and the clinical state of the transplanted kidney was somewhat disappointing. The only clear-cut result is the increased proportion of blast-like cells during rejection episodes. It had been reported that plasma cells were more abundant in late rejection, while lymphocyte and blast-like cells were found in a larger proportion in early rejection. Our results do not confirm these latter observations.

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