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Invited Review

Macrophages in the external muscle layers of mammalian intestines

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Summary. The literature on macrophages in the muscularis externa of mouse, rat, guinea pig, cat, dog and human gut is reviewed. In smaller mammals macrophages are regularly situated in two locations: in the serosa and at the level of Auerbach's plexus between the longitudinal and circular muscle layers. In addition a few solitary cells are present at the level of the deep muscular plexus. At the level of Auerbach's plexus the macrophages occur as a constant and regularly distributed cell population with intimate associations between macrophages and interstitial cells of Cajal. Morphologically they differ from most resident macrophages in being irregular in shape with 4-6 primary cytoplasmic processes, which branch and give a stellate appearance. They have been demonstrated with endocytotic markers (trypan red, FITC-dextran, cholera toxin), immunocytochemically with macrophage antibodies (F4/80, M1/70) and antibodies against MHC class-II antigen, GABA and cGMP. In muscularis externa of the human gut a regularly distributed cell population of macrophages is not obvious. However, a phenotypically distinct subgroup is identified by light microscopy with the pan macrophage antibodies (EBM11, C3b1 and partly by p150.95), and shows MHC class-II antigen. By electron microscopy muscularis externa macrophages, in all species investigated, appear to be endocytically downregulated, and since they are lysozyme, prostaglandine H synthase (both constitutive and activated) and acid phosphatase negative, they appear to be inactivated cells. Both origin and function of these cells are unknown. They may be immunocompetent, participate in a neuroimmune axis, tissue growth and modulation or other regulations of specific cell functions.

Key words: Macrophages, Small intestine, Colon, Interstitial cells of Cajal, Muscularis externa

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Introduction

Mononuclear phagocytes are found in almost all the major organs and tissues of the body. Macrophages have been shown to be functionally heterogeneous cells, which besides endocytosis are involved in a variety of different functions: in regulation of the specific immune response, in inflammation, in regulation of tissue growth and modeling, and in production of mediators that regulate other cells. In the intestines, macrophages have mainly been studied in the *mucosa* (LeFevre et al., 1979; Golder and Doe, 1983; Wilders et al., 1983; Winter et al., 1983; Hume, 1985; Sminia and Jeurissen, 1986; Hume et al., 1987; Sminia and van der Ende, 1988; van Rees et al., 1988; Mahida et al., 1989; Soesatyo et al., 1990; Pavli et al., 1993). In the *muscularis externa* three major cell types are present in the interstices: interstitial cells of Cajal, fibroblast-like cells, and macrophages (Taxi, 1965; Thuneberg, 1982, 1989). The early investigations of this tissue have been focused on the interstitial cells of Cajal (the putative pacemaker cell of the gut), but with progress of modern methods and increasing knowledge of macrophage functional heterogeneity the macrophages have become considered significant cells in this area. In the muscle layers the macrophages are present as a constant and regularly distributed cell population, with a close relationship to interstitial cells of Cajal (Thuneberg, 1982; Mikkelsen et al., 1985, 1988a,b). This is evident in smaller mammals (mouse, guinea pig), whereas a regular distribution of macrophages is not obvious in human intestines (Mikkelsen and Rumessen, 1992).

Historical background

The study of macrophages in the muscularis externa has emerged from the many studies of interstitial cells of Cajal, which were first described in the last part of the 19th century and have been the center of much debate due to different interpretations of morphological data

(Cajal, 1911; Taxi, 1965; Botar, 1966; Gabella, 1979; Thuneberg, 1982, 1989; Kobayashi, 1990). The denomination interstitial cells originates from Ramón y Cajal, who used a Golgi silver method and observed branched cells forming a network in the deep part of the circular muscle layer among other places (Cajal, 1893). Using methylene blue he described stellate cells with long ramified fibrillar extensions between the longitudinal and circular muscle layers (Cajal, 1911). Cajal believed these cells to be small «primitive» nerve cells, which besides the nerve plexus innervated the smooth muscle cells. Other investigators had different views. They proposed that the cells were (1) connective tissue cells which display special morphological characteristics (Dogiel, 1899; Cook and Burnstock, 1976; Gabella, 1979; Kobayashi, 1990), (2) Schwann cells of the terminal nerve plexus (Lawrentjew, 1926) and (3) pacemaker cells of the gastrointestinal tract (Keith, 1915; Thuneberg, 1982, 1989; Faussone-Pellegrini, 1987; Rumessen, 1994). The efforts to characterize ICC between the longitudinal and circular muscle layers caused several cell types to come to light , but interpretations differed widely. Ottaviani and Cavazzana (1940) injected trypan-blue into guinea pigs and observed cells with fine granules deposited especially around the nucleus and in the cytoplasmic processes. They believed these cells to be interstitial cells of Cajal, as they found that they could be distinguished from macrophages which contained much larger granules (Ottaviani et al., 1964). It is not clear from their pictures which cells they observed.

Feyrter (1951) used Ehrlich's acid hematoxylin and distinguished two main types of interstitial cells between the longitudinal and circular muscle layers, both having ramified processes. The cells were mainly distinguished by their nuclei. One had a small dark irregular nucleus (macrophage?) while the other had a large egg-shaped, light nucleus and a distinct nucleolus (ICC?).

In 1965 Taxi confirmed the coexistence of macrophages and interstitial cells of Cajal at the level of Auerbach's plexus using a double staining method: intraperitoneal injection of trypan red, followed by supravital methylene blue staining. By this technique it was possible to distinguish the two cell types both by light and electron microscopy and describe their distribution. However, in more recent papers based on electron microscopy and immunohistochemical methods these cells seem to have been overlooked or misinterpreted.

In guinea pig stomach, ileum, and colon Cook and Burnstock (1976) described "a small granule containing cell" external to nerve bundles within the region of Auerbach's plexus. It contained large, round, sometimes elongated vesicles with a large electron-dense core. A basal lamina was not present but the cell contained pinocytotic vesicles. In addition fibroblast-like cell processes were closely associated with these cells. The cells could well be macrophages. Cook and Burnstock, in tissue which showed axonal degeneration following 6-hydroxydopamine and 5,6-dihydroxytryptamine found these cells resistant to the treatment and found no indication of phagocytic activity.

In the connective tissue between the muscle layers of cat small intestine Taylor et al. (1977) described a cell type with many granules and shorter processes than other interstitial cells in the area. This was the only cell type to take up particles upon incubation in Krebs solution containing colloidal gold. Taylor et al. (1977) presumed it was a mast cell, but because of its endocytotic and accumulating properties it seems more likely that it was a macrophage.

In their study of the distribution and fine structure of the interstitial cells of Cajal Vajda and Fehér (1980) demonstrated these cells by light microscopy. However, the cells they showed by electron microscopy bear a strong resemblance to macrophages, rather than interstitial cells of Cajal.

In 1982 Thuneberg, while studying the interstitial cells of Cajal in muscularis externa of mouse small intestine, observed some other cells with ultrastructural features of macrophages. As there seemed to be a constant presence and close relationship between these

Table 1. Macrophages in small and large intestine.

	SMALL INTESTINE	COLON
Guinea pig		
Ottaviani and Cavazzana, 1940	+	
Taxi, 1965	+	
Cook and Burnstock, 1976	+	+
Mikkelsen et al., 1988b	+	
Kobayashi et al., 1989	+	
Zhou and Komuro, 1992	+	
Young et al., 1993	+	+
Anderson and Edwards, 1993	+	+
Mouse		
Rumessen et al., 1982	+	
Thuneberg, 1982	+	
Mikkelsen et al., 1985	+	
Faussone-Pellegrini, 1985	+	
Mikkelsen et al., 1988a	+	
Mikkelsen et al., 1988b	+	
Anderson and Edwards, 1993	+	+
Rat		
Komuro, 1989	+	
Anderson and Edwards, 1993	+	+
Cat		
Taylor et al., 1977	+	
Vajda and Fehér, 1980	+	
Dog		
Torihashi et al., 1994		+
Man		
Faussone-Pellegrini et al., 1990		+
Rumessen and Thuneberg, 1991	+	
Rumessen et al., 1992	+	
Mikkelsen and Rumessen, 1992	+	+
Rumessen et al., 1993	+	

two cell types, we started to characterize them in mouse, guinea pig and human intestines (Mikkelsen et al., 1985, 1988a,b, 1990, 1991; Mikkelsen and Rumessen, 1992; Rumessen et al., 1982, 1992, 1993).

In later studies the presence of macrophages has often been overlooked. In an EM study of the cytodifferentiation of interstitial cells of Cajal related to mouse myenteric plexus Faussone-Pellegrini (1985) described macrophages and fibroblasts in the adult mouse intermingled with interstitial cells of Cajal, or preferentially situated near the blood vessels. Macrophages were not mentioned in foetuses, unfed neonates (where ICC and fibroblasts were observed), suckling animals (3-14 days), nor weaning animals. However, in 5-day-old Balb/c mice cells capable of FITC-dextran uptake are present (Mikkelsen, unpublished observation).

Kobayashi et al. (1989) observed immunoreactivity for gamma-aminobutyric acid (GABA) in cells at the level of Auerbach's plexus of guinea pig. At the time they interpreted the cells as fibroblast-like cells (i.e. interstitial cells of Cajal), but the cells observed had broader unconnected processes with irregular outlines, and as such are very similar to macrophages demonstrated with FITC-dextran endocytosis or by macrophage markers (Mikkelsen et al., 1985, 1988a,b;

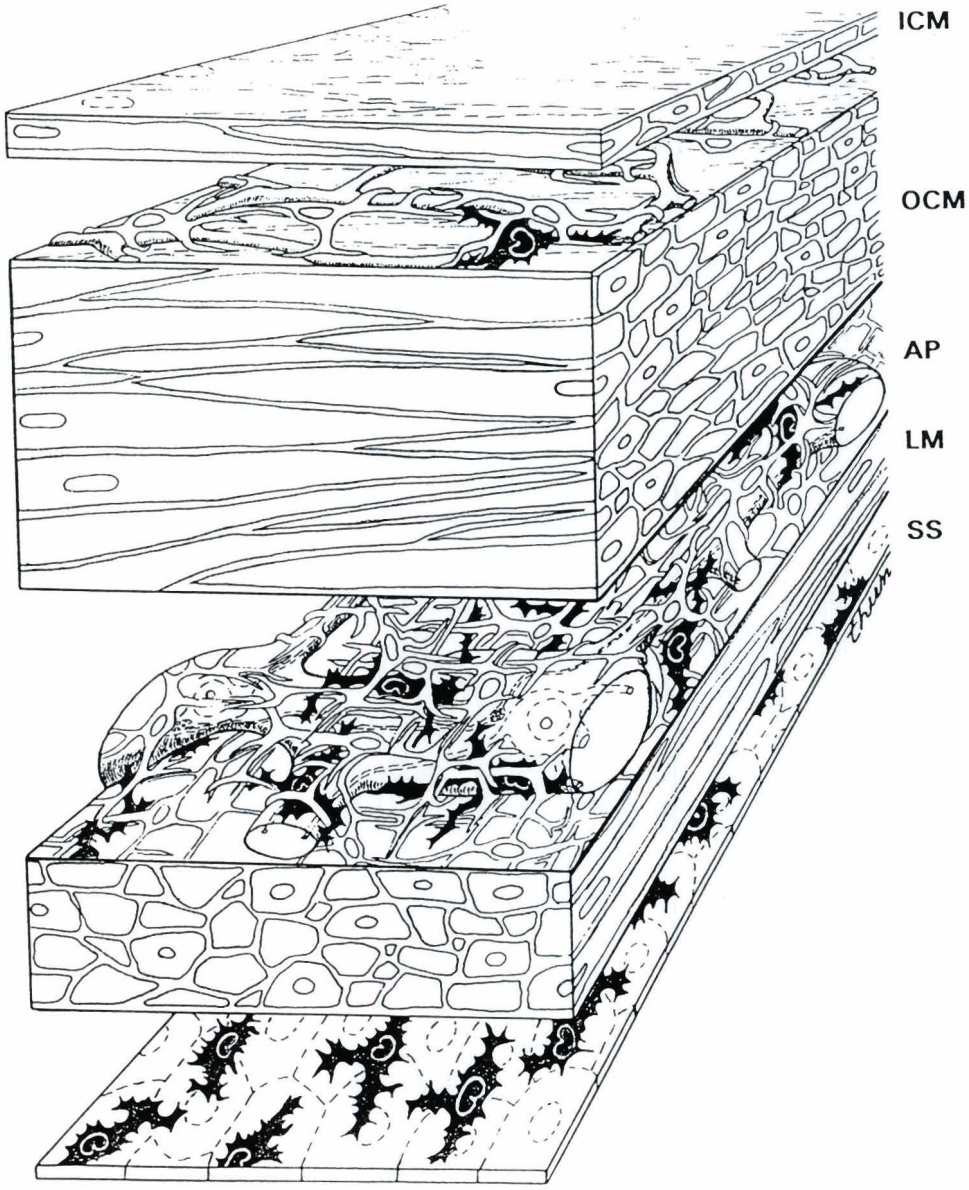


Fig. 1. Localization of macrophages in the muscularis externa and serosa of smaller mammals. Macrophages (black cells) are situated in the serosa (SS), between the longitudinal (LM) and outer circular muscle layer (OCM) at the level of Auerbach's plexus (AP) in close contact with interstitial cells of Cajal (a network of white cells). A few macrophages are present at the deep muscular plexus between the outer circular muscle layer and the inner circular muscle layer (ICM). Drawing by L. Thuneberg.

Young et al., 1993). Since the GABA immunoreactivity of the cells is strongest after incubating specimens with GABA, the cells are most likely macrophages, which have endocytosed the GABA both in vivo and in vitro.

Komuro (1989) distinguished three types of cells between the muscle layers of rat intestine; (1) a fibroblast-like cell was the most common cell type, (2) a second cell type was characterized by the presence of many mitochondria and was considered to be an interstitial cell of Cajal, and (3) a third cell type was characterized by the presence of large granules and/or vacuoles, together with many coated vesicles and was thought to be a macrophage. Moreover Zhou and Komuro (1992) described macrophages in association with the deep muscular plexus in guinea pig small intestine as an infrequently observed cell type.

In a recent report Young et al. (1993) showed basal cyclic GMP immunoreactivity in macrophages of guinea pig ileum and colon; the positive cells were situated in the serosa as well as at the level of Auerbach's plexus. This identification of the cells as macrophages was confirmed by double-labeling experiments using the macrophage antibody F4/80 or intravenously injected FITC-dextran.

While trying to label interstitial cells of Cajal Anderson and Edwards (1993) found that cholera toxin b in mouse and rat labels interstitial cells of Cajal, neurons, glia cells and macrophages, whereas in guinea

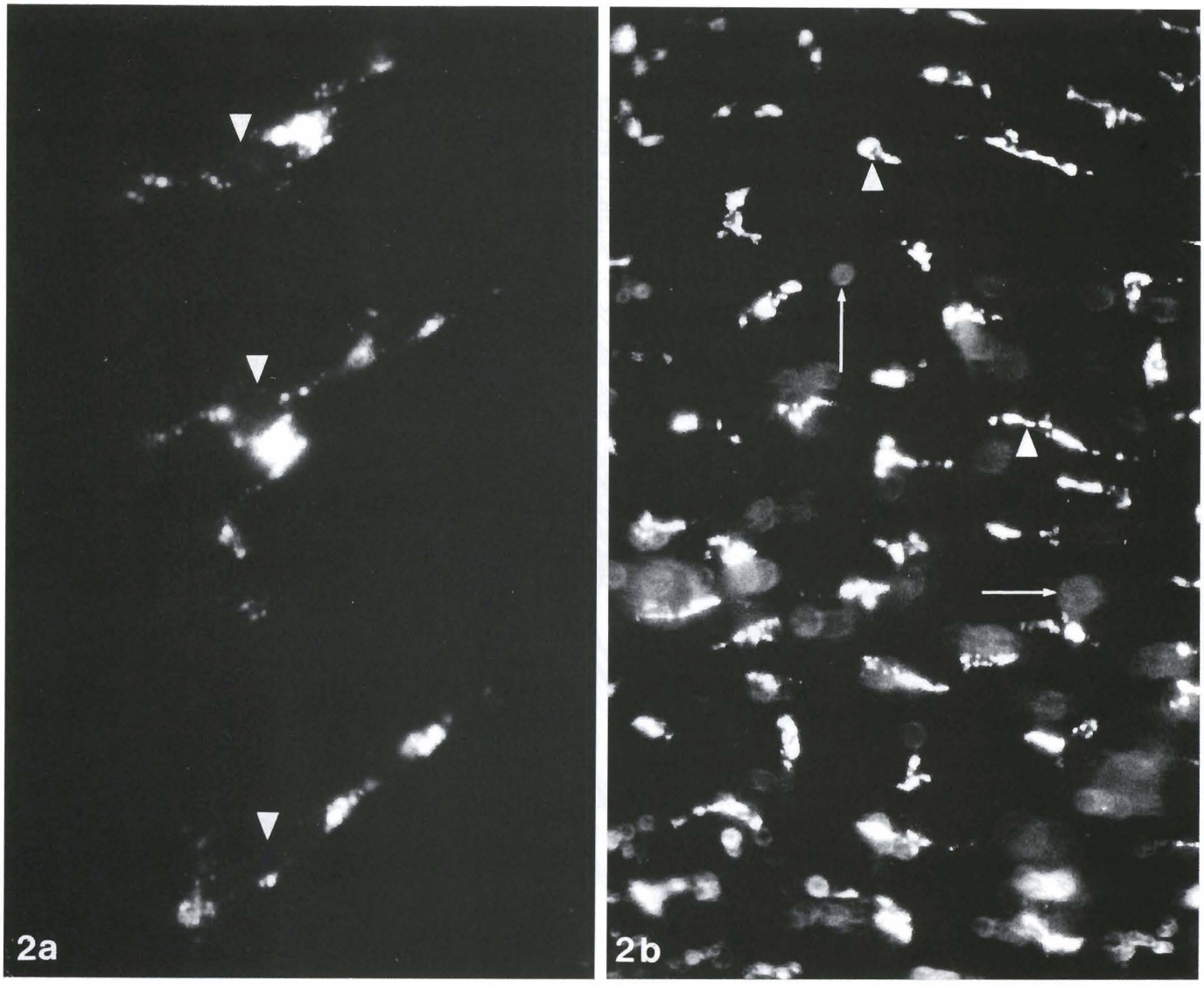


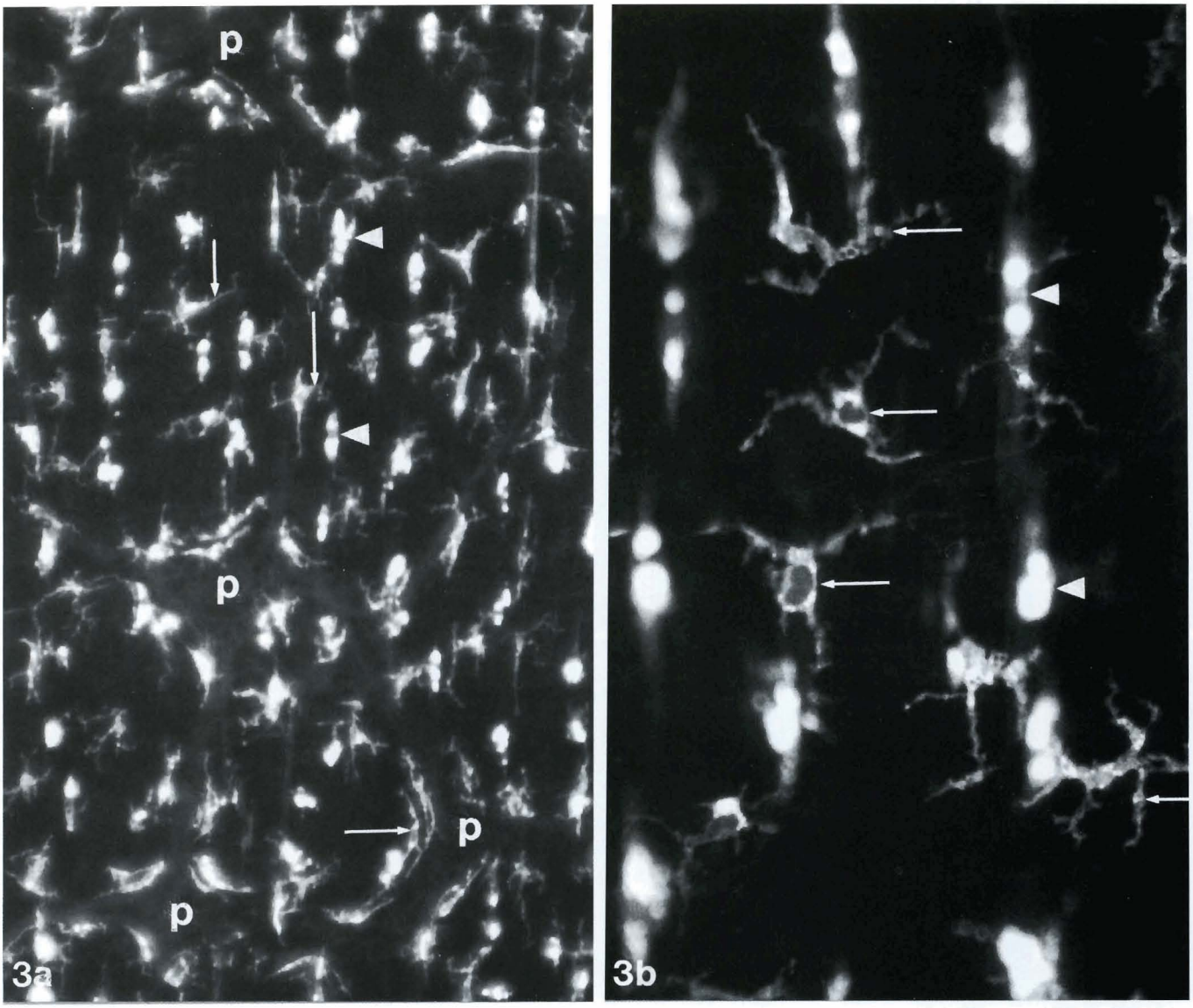
Fig. 2. Mouse **a.** Fluorescence micrograph of isolated musculature 1 day after dextran administration. Serosal macrophages are rather regularly distributed, with numerous fluorescent granula and a few long slender cell processes. Negative image of nuclei (arrowheads). x 875. **b.** Fluorescence micrograph of isolated musculature 4 days after dextran administration. Serosal macrophages (arrowheads) arranged in rows. Macrophages at Auerbach's plexus (arrows) are out of focus. x 220. From Mikkelsen et al. 1985. Copyright Wiley-Liss Inc.

pig cholera toxin labeled only macrophages, neurons and enteric glia cells. Cholera toxin b is believed to bind to a cell surface receptor, the ganglioside G_{M1}. Macrophages may express G_{M1}, but since they are actively endocytosing cells and the labeling consists of a faint general staining of the cytoplasm and a distinct staining of granules 1-2 µm in diameter, an endocytic origin is not excluded. In human colon Faussone-Pellegrini et al. (1990) described macrophages at the level of the finer nerve endings, and in dog colon Torihashi et al. (1994) described macrophages at the level of Auerbach's plexus. A survey of papers (Table 1) includes macrophages in muscularis externa shown or described with or without the recognition by the authors.

Morphology

Light microscopy

In smaller mammals it has been possible to study the distribution and endocytotic capacity of macrophages in the small intestine by using FITC-dextran as an endocytotic tracer in combined fluorescence stereo microscopy, fluorescence microscopy and electron



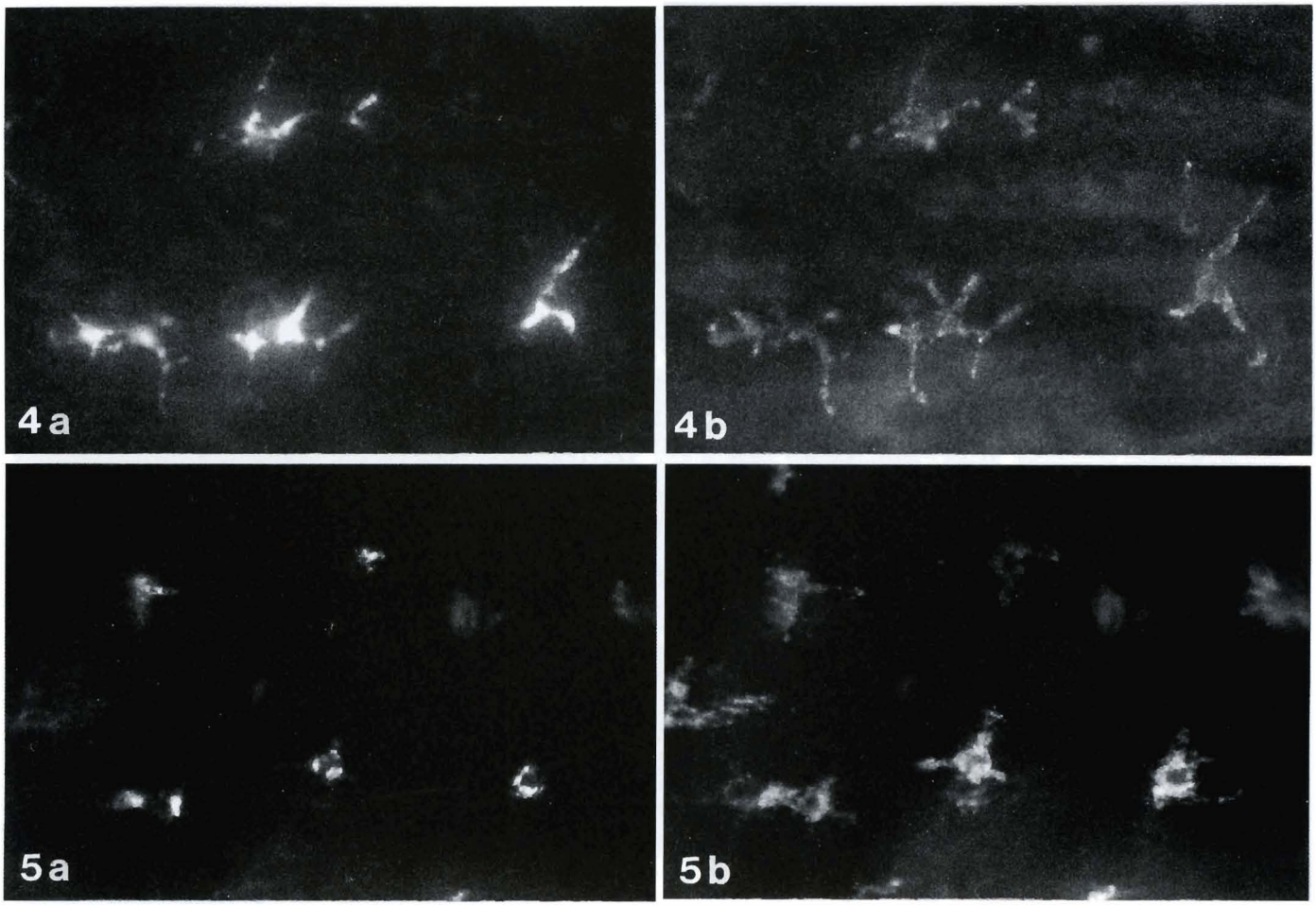
Figs. 3. Mouse. **a.** Fluorescence micrograph of isolated musculature 1 day after FITC-dextran administration. The macrophages between the two muscle layers (arrows) are evenly distributed, and some are lining Auerbach's plexus (p). Serosal macrophages (arrowheads) are slightly out of focus and arranged in rows. x 130. **b.** Fluorescence micrograph of isolated musculature 1 day after dextran administration. The macrophages between the two muscle layers (arrows) have many branching cytoplasmic processes. Serosal macrophages (arrowheads) are slightly out of focus. x 700. From Mikkelsen et al. 1985. Copyright Wiley-Liss, Inc.

microscopy (Mikkelsen et al., 1985). The macrophages are distributed in the serosa close to the longitudinal muscle layer, at the level of Auerbach's plexus between the longitudinal and circular muscle layers, and at the level of the deep muscular plexus between the outer and inner circular muscle layer (Fig. 1) (Mikkelsen et al., 1985; Young et al., 1993).

Macrophages vary morphologically depending on their location in mouse and guinea pig muscularis externa. The **serosal** macrophages are slender oblong cells 50-90 µm by 5-20 µm, with a centrally situated nucleus, which is often indented (Fig. 2a,b). They have 3-5 long, slender, sometimes bifurcated cytoplasmic processes. The cell bodies and main processes are orientated in rows parallel with the longitudinal muscle cells (Mikkelsen et al., 1985, 1988a; Young et al., 1993). The macrophages **at the level of Auerbach's plexus** are more evenly distributed and seem more irregular in shape than serosal cells (Fig. 3a,b). They are 40-90 µm by 5-15 µm and the nuclei are oval, often indented, and situated centrally in the cells. Most cells have 4-6 primary cytoplasmic processes, which branch further to give a stellate appearance, with small protrusions along the processes. This description is based on studies of wholemounts with FITC-dextran labeled macrophages (Mikkelsen et al., 1985), as well as preparations treated with macrophage surface antibodies (F4/80, IE, M1/70) (Figs. 4a,b, 5a,b) (Mikkelsen et al., 1988a).

By combining FITC-dextran uptake with supravital methylene blue staining it has been possible to demonstrate the regular distribution of both macrophages and interstitial cells of Cajal along the entire small intestine (Figs. 6a,b,c) (Mikkelsen et al., 1988b), and in addition, to confirm the constant intimate association between these two cell types noticed by electron microscopy (Thuneberg, 1982; Mikkelsen et al., 1985).

At the level of Auerbach's plexus macrophages can be distinguished from interstitial cells of Cajal by their broader processes which are more irregular in outline,



Figs. 4 and 5. Mouse. Fluorescence micrographs of wholemounts from duodenum (4a, 5a), showing cells containing FITC-dextran; (4b, 5b) the same cells after immunohistochemical staining. (4b) Serosal macrophages with F4/80 antigen, x 390. (5b) Serosal macrophages expressing IE antigen, x 300. From Mikkelsen et al. 1988a. Copyright Springer.

while the much more numerous interstitial cells of Cajal have cell processes which are longer, slender and join to form a network.

Macrophages at the **deep muscular plexus** are stellate cells but rather infrequent (Mikkelsen et al., 1988a).

The morphology of **human** macrophages in muscularis externa has not been investigated in wholemounts. The appearance in sections is similar to the one described above, the macrophages being rather large cells with irregular contours and several long cytoplasmic processes (Fig. 7). Human serosal macrophages appear rounder and less ramified (Mikkelsen and Rumessen, 1992).

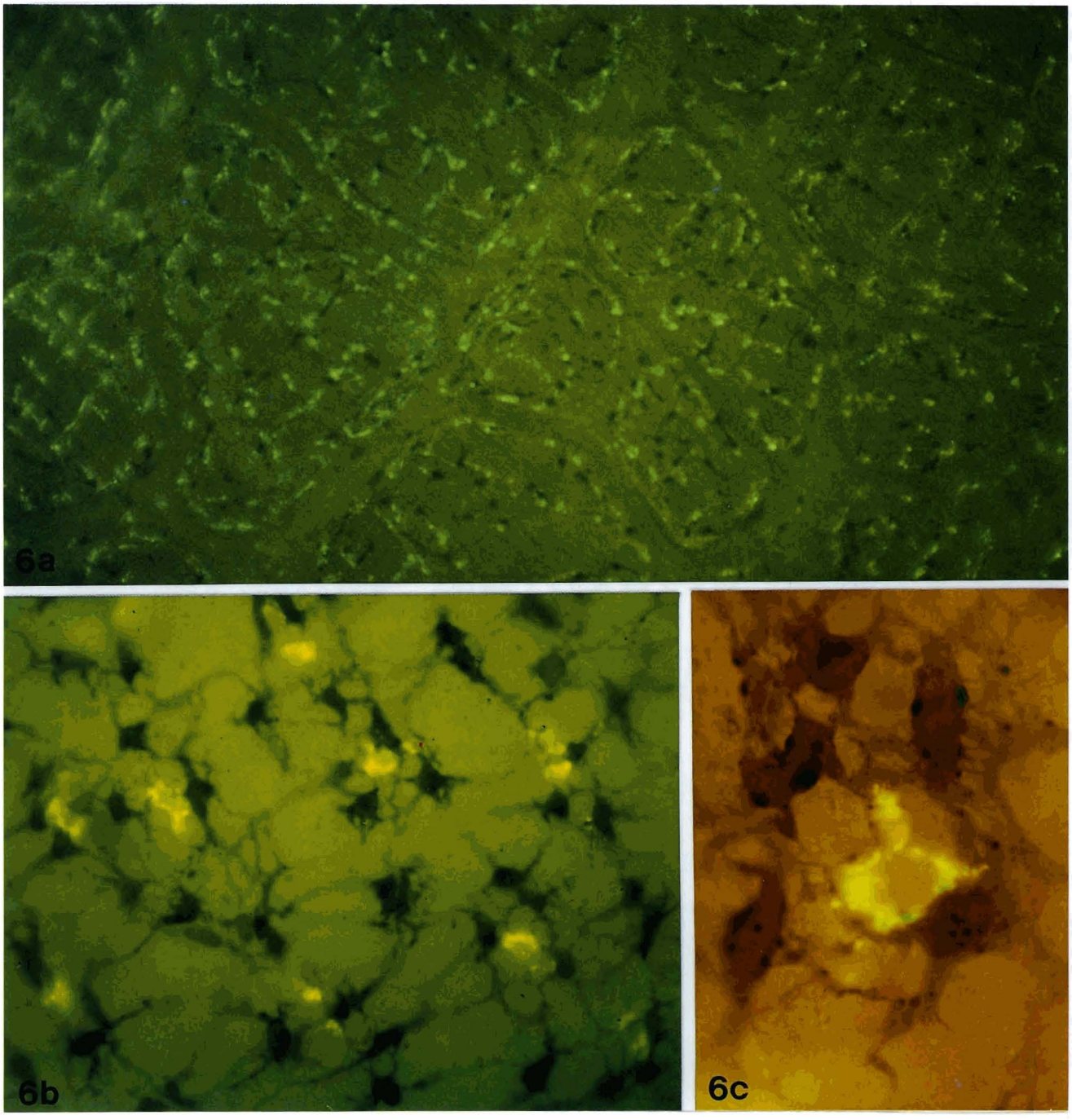


Fig. 6. Whole-mounts of intestinal wall, double-labeling technique: FITC-dextran uptake by macrophages and methylene blue staining of ICC. **a.** Guinea pig duodenum. x 100; **b.** Adult mouse ileum. x 400. **c.** Adult mouse duodenum. x 900. From Mikkelsen et al. 1988b. Copyright Springer.

Mononuclear phagocytes can adopt a wide range of shapes depending upon location in a given tissue. The resident macrophages of the brain, the microglia, provide an example of such specialization. The morphology of microglia varies characteristically with the region (Perry and Gordon, 1989; Lawson et al., 1990); in the gray matter they are small cells from which a number of fine branches arise and divide to give a radial ramified appearance. In the circumventricular organs they appear as compact cells, smaller, but similar to Kupffer cells. They appear to be quiescent cells as judged by their ultrastructure and antigenic phenotype (Gordon et al., 1988; Perry and Gordon, 1991). Another arborized cell type is the regularly dispersed Langerhans cell situated within epithelia (Gordon et al., 1992).

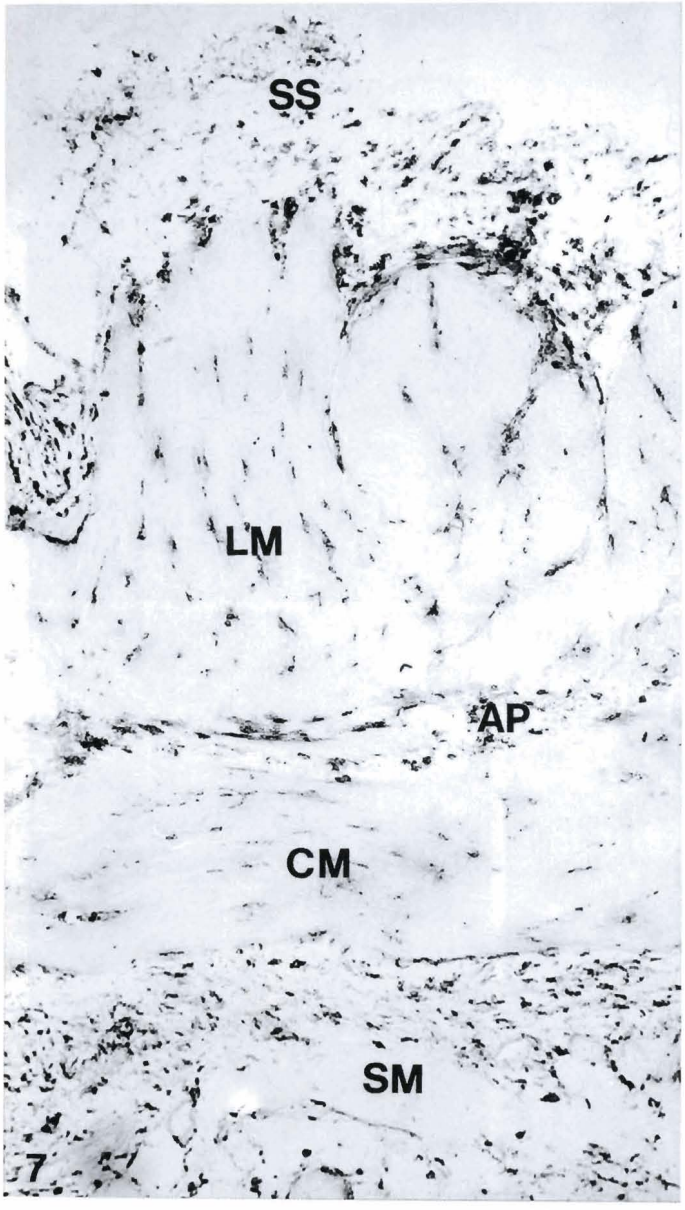


Fig. 7. Man. Ileum. CD68 immunoreactivity of cells in serosa (SS), in the longitudinal muscle layer (LM), at Auerbach's plexus (AP), in the circular muscle layer (CM) and in submucosa (SM). x 80. From Mikkelsen and Rumessen, 1992. Copyright Springer.

Electron microscopy

It is characteristic that macrophages in the different locations of the muscularis externa contain many vesicles and vacuoles of variable size, shape and content (Figs. 8, 9). Especially dense bodies with the features of lysosomes are conspicuous. Another distinguishing feature of the cell periphery is the presence of many coated vesicles and pits. In contrast to interstitial cells of Cajal the macrophages have an indented and more electron dense nucleus, and contain few and small mitochondria. The cytoplasm contains scattered strands of rough endoplasmic reticulum. The cell processes appear short and blunt and lack the cytoplasmic specializations of the processes of the interstitial cells of Cajal (filament bundles, smooth endoplasmic reticulum, caveolae). Under normal circumstances macrophages show a restricted or low phagocytic ability (Mikkelsen et al., 1985).

In mouse the ultrastructure of macrophages at the site of Auerbach's plexus appears similar to that of macrophages in the serosal layer, although they exhibit fewer vesicles and more cell processes. In mouse at the level of Auerbach's plexus macrophages are generally in close contact with or enveloped by processes from interstitial cells of Cajal, while serosal macrophages often are enveloped by processes of fibroblast-like cells. At the deep muscular plexus they are often enveloped by processes of ICC or fibroblast-like cells (Figs. 10-12) (Rumessen et al., 1982; Thuneberg, 1982).

In man, serosal macrophages appear richer in secondary lysosomes (Fig. 13). Human macrophages within the muscle layers (in septa between muscle lamellae and in intralamellar septa) are in close contact with fibroblasts and occasionally with nerves (Fig. 14) (Mikkelsen and Rumessen, 1992; Rumessen et al., 1993).

Phenotype

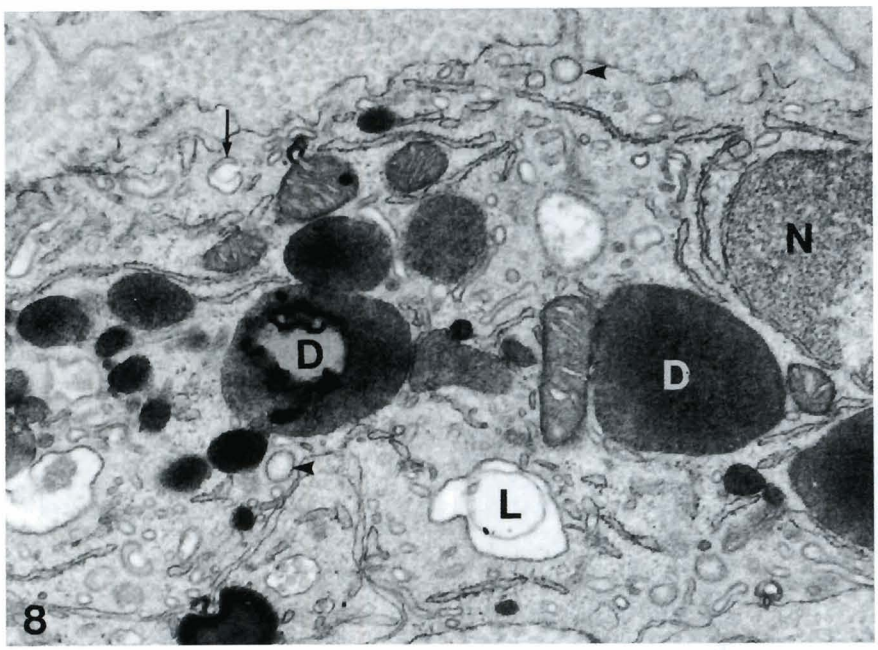
Because of the complexity of the mononuclear phagocyte and antigen presenting cell system, there is a great interest in identifying marker antigens that can be used to characterize subsets of these cells. Monoclonal antibodies which react with subpopulations are presumed to disclose distinct functions of the cell (Hogg, 1987). Differential binding of antibodies can be used to determine the different phenotypes of macrophages. Antibodies against macrophages are available for most species.

A rat monoclonal antibody F4/80, which is directed to a plasma membrane glycoprotein with unknown function (Austyn and Gordon, 1981), marks most macrophages in mouse (Fig. 4b).

Commonly used macrophage markers in rat are ED1, ED2 and ED3 (Dijkstra et al., 1985).

In man EBM11 antibodies recognize cells included in the classic monocyte/macrophage system and belong to the CD68 group in the white cell differentiation antigen system (Figs. 7, 15a) (Kelly et al., 1988; Micklem et al., 1989). Antibodies against CR3 complement receptors (type III complement receptor) exist for both mouse and man (M1/70 and C3bi). These receptors are considered important for phagocytosis.

Another group of antibodies detects cells which express the major histocompatibility complex class-II antigen (MHC class-II antigen) (in mouse I-E/Ia, and in man HLA-DR) (Figs. 5b, 15b); these receptors are considered essential for a cell to present antigen to a T lymphocyte. MHC class-II antigen positive cells are usually referred to as antigen-presenting cells. Only activated cells should be MHC class-II antigen positive. However, in mouse MHC class II antigens have been described as a constitutive trait of resident mononuclear phagocytes in most sites of the body (Hume, 1985). In mouse most macrophages of the muscularis externa identified with FITC-dextrans show immunoreactivity for F4/80, M1/70 and Ia-antigen (Mikkelsen et al.,



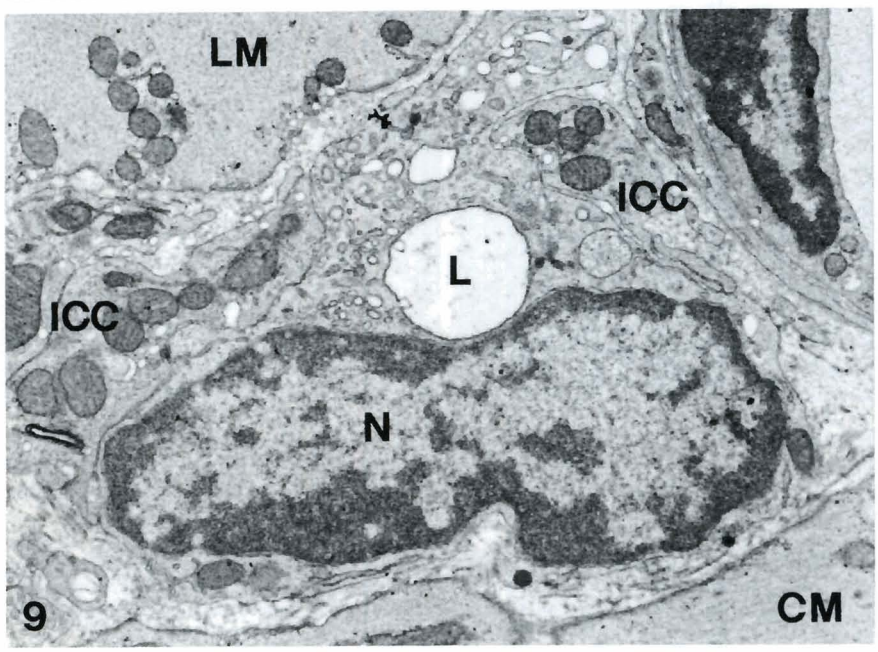
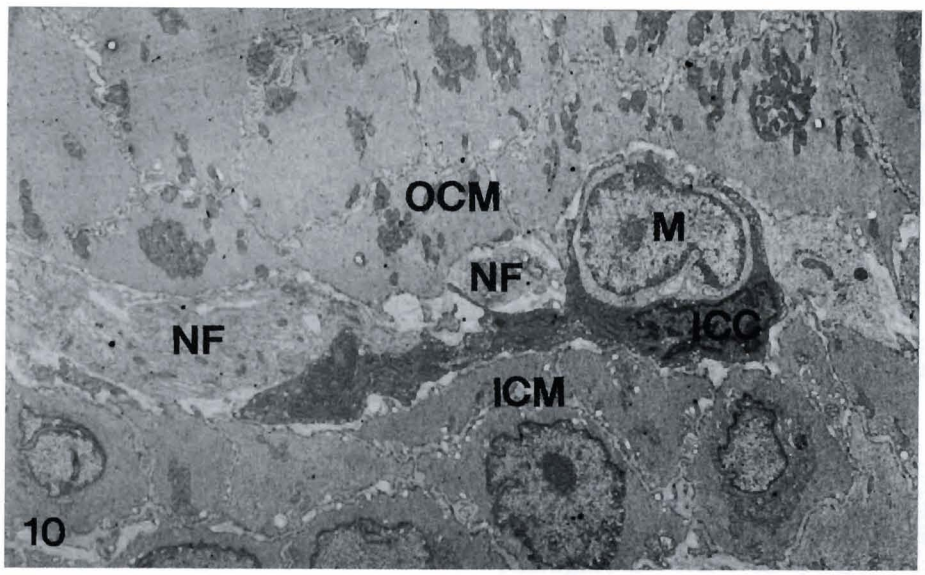
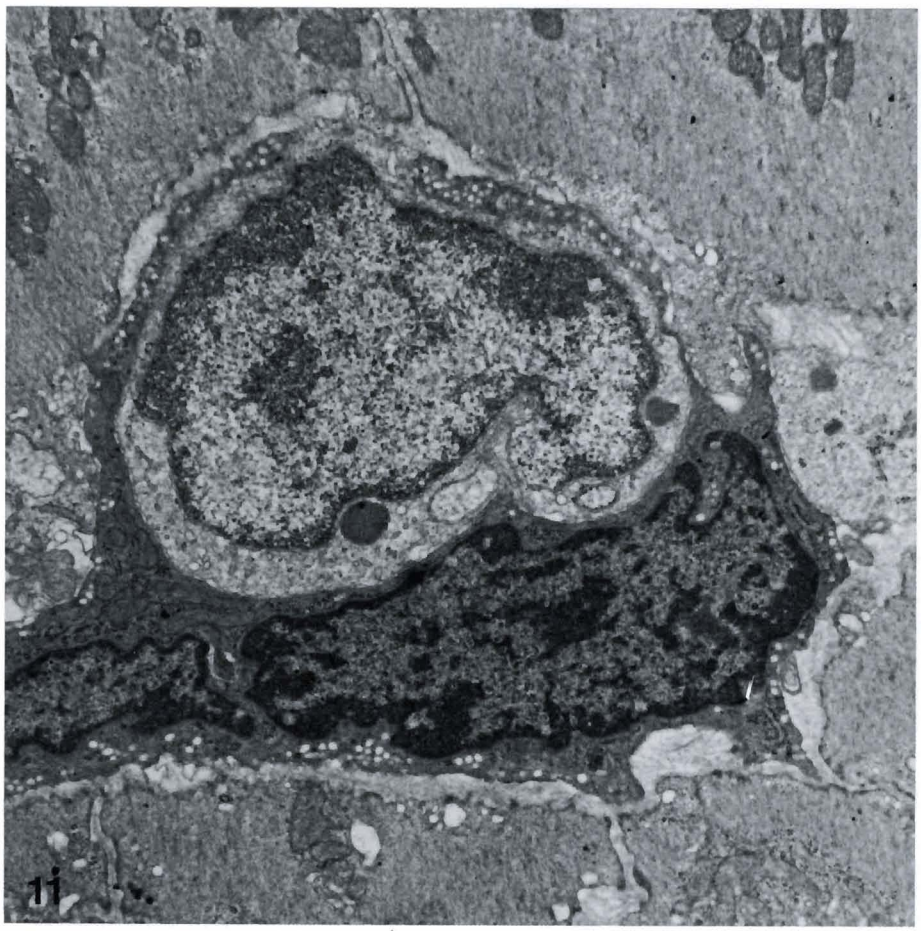
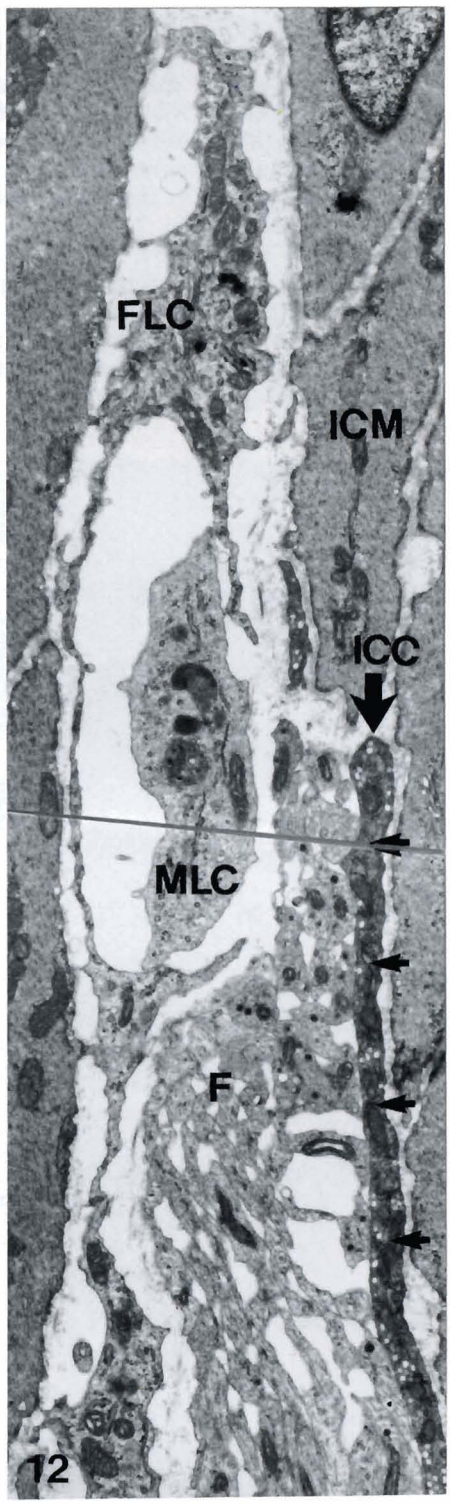


Fig. 8. Mouse. Macrophages in the serosal layer with light vesicles (L), many dense bodies (D), coated vesicles (arrowheads), doughnut-shaped vesicles (arrow), and nucleus (N). x 30,000.

Fig. 9. Mouse. Macrophages at the level of Auerbach's plexus enveloped by cell processes from interstitial cells of Cajal (ICC). Small, dense bodies and numerous coated vesicles are present. L: light vesicles; N: nucleus; LM: longitudinal muscle layer; CM: circular muscle layer. x 15,000. From Mikkelsen et al. 1985.







Figs. 10 and 11. Mouse. Morphology and topology of ICC and macrophages (M) associated with the deep muscular plexus (PMP) between the outer (OCM) and the inner circular (ICM) muscle layers. A nerve fasciculus (NF) of PMP is accompanied by two cell types, ICC with dense cytoplasm and nucleus, and macrophages with light cytoplasm and large, dense cytoplasmic granules. The nucleated part of the macrophage is closely enveloped by processes of an ICC. Fig. 10. x 3,900; Fig. 11. x 15,000. From Thuneberg, 1982. Copyright Springer.

Fig. 12. Mouse. The deep muscular plexus and interstitial cell types in a preparation with experimentally induced edema. All structures are widely separated except ICC and some naked terminal profiles which remain in close (10-20 nm) contact (arrows). Fibroblasts (FLC) seem to envelop macrophage (MLC) and larger fasciculi of PMP, but are not innervated by terminal profiles (F). The inner lamina of circular muscle (ICM) is completely shielded from terminals of PMP by means of the long flattened ICC processes. x 12,900. From Rumessen et al. 1982. Copyright Wiley-Liss, Inc.

1988a).

In man we were unable to demonstrate macrophages by endocytosis of FITC-dextran. However, by using *immunohistochemical methods* it is possible to describe their localization and distribution (Mikkelsen and Rumessen, 1992). CD68-positive cells are situated in the serosa, the muscle layers and in the submucosa (Fig. 7). Some of the positive serosal cells appear to line the longitudinal muscle layer. CD68-positive cells are located in septa between the main muscle lamellae, in smaller intralamellar septa between the muscle cells, and at the level of Auerbach's plexus, where they line the ganglia. CD68 positive macrophages are C3bi positive. In the muscle layers CD68-positive cells are MHC-class-II antigen positive (Fig. 15a), in contrast to cells in serosa and submucosa. In lamina propria from human intestine Hume et al. (1987) described a phenotypically different macrophage which is MHC class-II antigen positive, CD4 positive, and OKM1-negative (type III complement receptor). Nibbering et al. (1987) have suggested that differences in macrophage phenotypical and morphological

Table 2. Histological methods used to identify macrophages in the external muscle layers.

	MOUSE	RAT	GUINEA PIG	MAN
Endocytosis				
	FITC-dextran cholera toxin	FITC-dextran cholera toxin	FITC-dextran cholera toxin trypan red trypan blue	
Immunomethods				
	F4/80 M1/70 Ia		F4/80 c-GMP GABA	CD68 C3bi HLA-DR

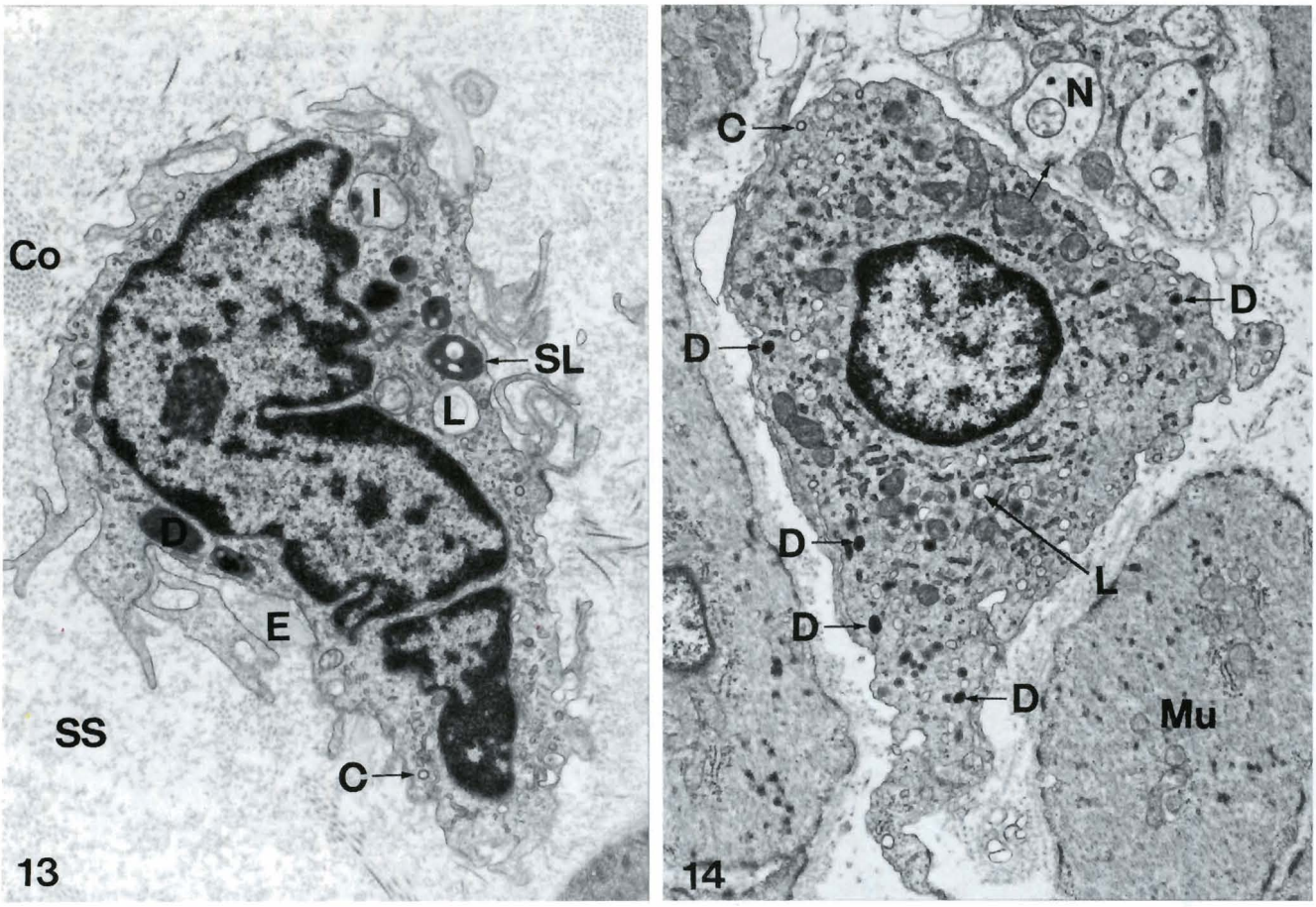


Fig. 13. Man. Ileum. Serosal macrophage containing numerous dense vesicles (D), secondary lysosomes (SL), light vesicles (L), intermediate vesicles (I), and coated vesicles (C). The cell is surrounded by elastic fibers (E) and collagen fibers (Co). SS: serosa. x 7,900.

Fig. 14. Man. Ileum. Macrophage in an intralamellar septum, surrounded by muscle cells (Mu). Naked nerve terminals (N) are close to the cell; a density of presynaptic membrane is apparent (arrow). The macrophage contains many dense (D), light (L), and coated (C) vesicles. x 10,400. From Mikkelsen and Rumessen 1992. Copyright Springer.

appearance could depend on their location (micro-environment). Table 2 shows the different markers used in macrophage identification.

Origin and differentiation

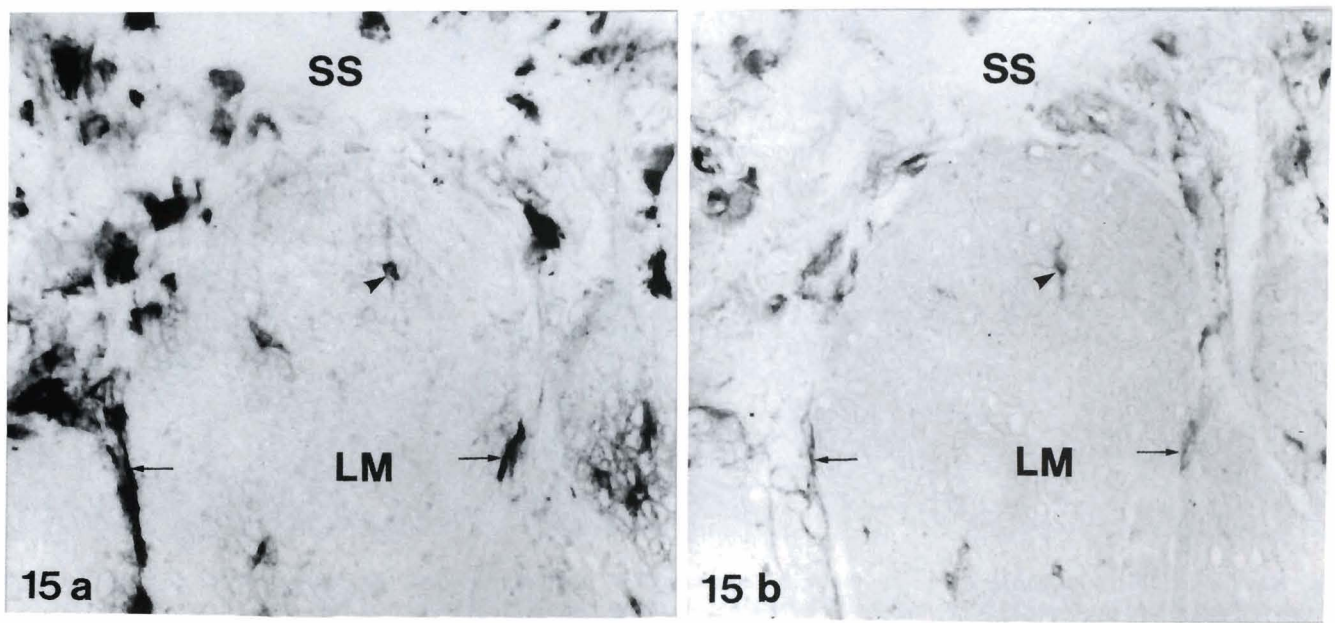
The origin and differentiation of the muscularis externa macrophages have not been examined. However, they should be considered resident macrophages, as they occur in the absence of an exogenous or endogenous inflammatory stimulus. In addition, activated macrophages (1) and elicited macrophages (2) are defined as: (1) macrophages with increased functional activity induced by a given stimulus; activation implies an increase in one or more functional activities of a cell or a new functional activity, and (2) mononuclear phagocytes (macrophages) attracted to a given site by a given substance (an immunologically non-specific inflammatory stimulus); the term does not indicate the developmental stage or functional state of the cells (van Furth, 1989; Gordon et al., 1992). Unlike resident macrophages which show considerable regional heterogeneity, elicited and immunologically activated macrophages display a similar phenotype in different sites. Heterogeneity in phenotype and receptor expression between recruited and resident macrophages can be ascribed to differences in cell maturity and their modulation by lymphokines and other regulatory agents.

The origin of resident macrophages has been discussed in relation to other tissues. One theory is that they arise solely by self-replication and do not differentiate from monocytes (Naito, 1993), while another prevailing theory is that macrophages originate in bone marrow as monocyte precursors, enter the circulation as monocytes, and migrate from the vascular lumen to the tissues (liver, spleen and lung) and body cavities, where they differentiate into macrophages. A small proportion (5%) of the macrophages do derive from locally dividing mononuclear phagocytes, which are also bone marrow derived (van Furth, 1989). It is unknown whether the macrophages die in the tissues or migrate to local lymph nodes. Monocytes and monocyte-derived macrophages are considered to be short-lived, (turnover time: Kupffer cells 5-6 days, peritoneal macrophages 14.9 days) (van Furth, 1989). In rat foetus macrophages are reported to occur in the submucosa from day 18 (van Rees et al., 1988). Macrophages in the muscle layers could be long-term residents since we have observed cells containing large FITC-dextran vesicles in mice 7 weeks after exposure to the marker. In addition, mitotic figures in muscularis externa are rare (Mikkelsen, unpublished observation).

Function

Subpopulations may be distinguished not only by their state of differentiation as shown by histochemical and biochemical methods, but also by their functional activity. Macrophages have been reported to have a multitude of functions: endocytosis, antigen presenting, and secretion of a large variety of secretory products (among other lysozyme, prostaglandins, nitric oxide (NO), interleukin-1 and growth factors) (Nathan, 1987).

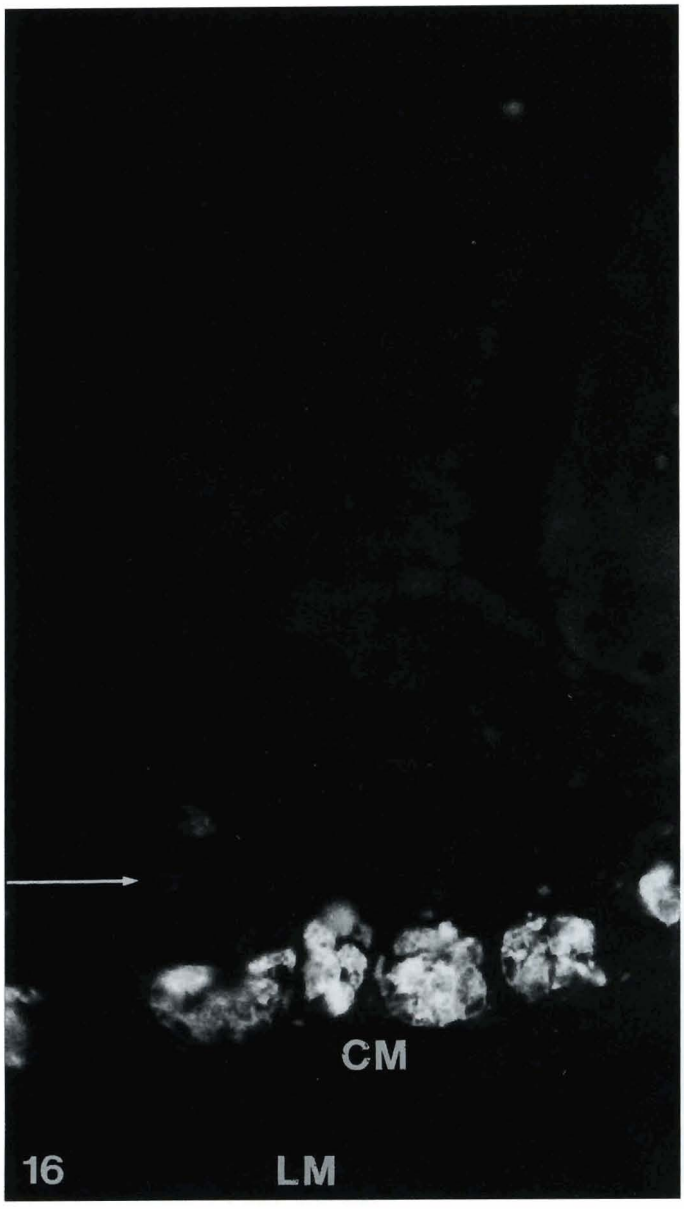
Actually, very little is known about the function of resident macrophages, since most functional studies have been done with in vitro macrophages (peritoneal,



Figs. 15. Man. Ileum, serial sections. **a.** CD68 positive cells in the outer part of the longitudinal muscle layer (LM). The reaction is very strong in cells lining the longitudinal muscle layer and in septa (arrows) and weaker in cells in intralamellar septa (arrowhead) serosa (SS). **b.** HLA-DR-positive cells, which all appear to be CD68-positive. x 330. From Mikkelsen and Rumessen 1992. Copyright Springer.

alveolar or macrophages in culture), which often have been elicited or immunologically activated making a direct comparison with a possible function of resident macrophages problematic. Macrophages in the muscularis externa could have several functions: endocytosis of the substantial amount of neuropeptides released by the nerves, regulation of tissue growth and modeling, regulation of the specific immune response in inflammation, and production of mediators that regulate other cells (motility?).

The production of lysozyme was generally regarded as a constitutive function of macrophages (Gordon et al., 1974). In mouse muscularis externa macrophages we did not find lysozyme immunoreactivity (Fig. 16) (Mikkelsen et al., 1988a). This could be due to lack of intracellular storage capacity. However, it has later been reported that in resting macrophages of most tissues (including mouse small intestine) it has not been



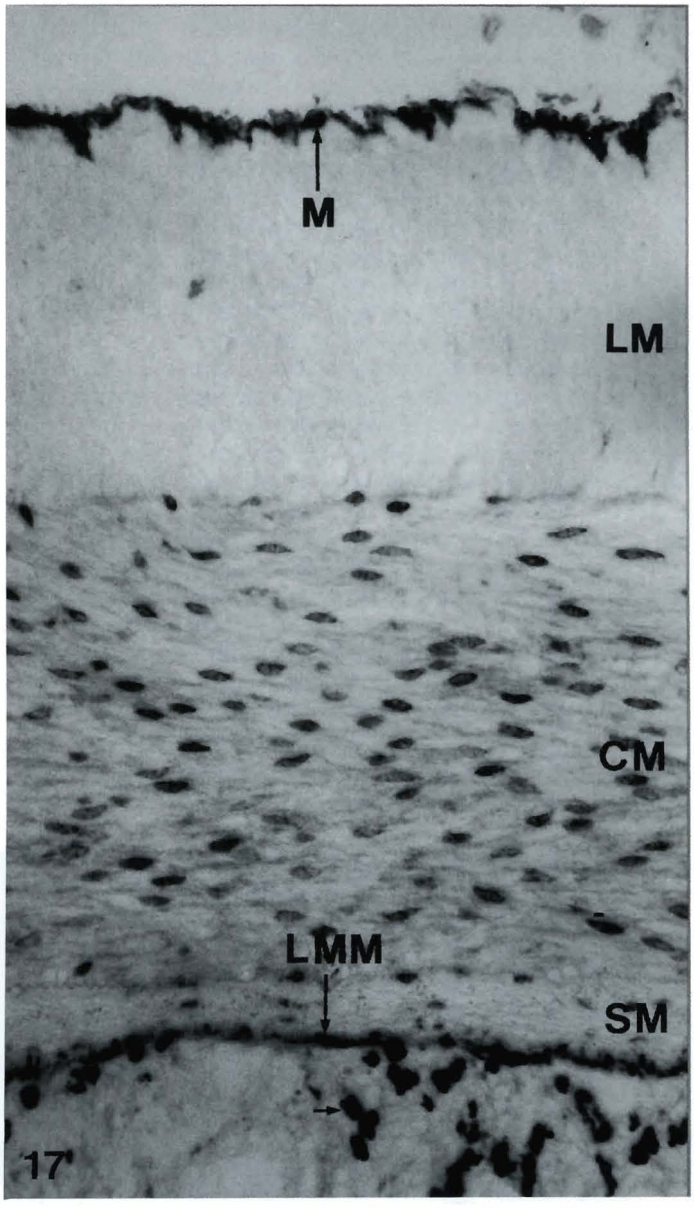


Fig. 16. Mouse. Fluorescence micrograph of a cryostat section from the small intestine. Antilysozyme labelled Paneth cells and a few cells in lamina propria (arrow). CM: circular muscle layer; LM: longitudinal muscle layer. x 300. From Mikkelsen et al. 1988a. Copyright Springer.

Fig. 17. Rabbit. Ileum, muscularis externa. Staining of PGH synthase in mesothelial cells (M), circular muscle layer (CM) and lamina muscularis mucosae (LMM). In lamina propria endogenous peroxidase containing cells (arrow) are heavily stained. The longitudinal muscle layer (LM) is unstained. SM: submucosa x 230. From Mikkelsen et al. 1990. Copyright Springer.

possible to detect lysozyme mRNA by in situ hybridization (Chung et al., 1988; Keshav et al., 1991), and that lysozyme expression is an indication of macrophage activation in vivo (Keshav et al., 1991).

Under some circumstances macrophages have been shown to be a source of prostaglandins (Page et al., 1978; Nathan, 1987), and prostaglandin biosynthesis has been demonstrated in all parts of the gastrointestinal tract (Ferreira et al., 1976; Bennett et al., 1977; LeDuc and Needleman, 1979). Prostaglandins and inhibitors of prostaglandins have been reported to have striking effects on mechanical and electrical activity (contractions and slow wave shape, amplitude and frequency) of the gastrointestinal musculature (Sanders and Ross, 1978; Coburn, 1980; Bennett et al., 1981; Sanders, 1984).

Since we had previously suggested that the macrophages might release prostaglandins, we examined the cellular origin of prostaglandin synthesis by immunohistochemical methods, using an antibody against the prostaglandin H synthase (Mikkelsen et al., 1990, 1991). We studied guinea pig, rabbit and human small intestine and human colon and found a species variation in the distribution of recognizable levels of prostaglandin H synthase immunoreactivity. Moreover, macrophages in the muscle layers appeared negative in all species. In guinea pig small intestine all muscle layers were unstained, with only mesothelial and endothelial cells positive. In rabbit small intestine the smooth muscle cells in the circular muscle layer and lamina muscularis mucosae were positive, in the longitudinal muscle layer negative (Fig. 17). In the human intestines prostaglandin H synthase immunoreactivity was present in most smooth muscle cells in the circular and longitudinal muscle layers as well as in lamina muscularis mucosae. Inducible prostaglandin synthase has later been isolated and should be produced by activated macrophages (Lee et al., 1992). However, using antibodies against inducible prostaglandin synthase (PG26) immunoreactivity in macrophages in normal mouse and rat muscularis externa was not apparent (Mikkelsen, unpublished observation).

In conclusion, macrophages of the muscularis externa may not contribute significantly to the production of prostaglandin under normal circumstances, and macrophages of muscularis externa appear in this respect to be unactivated cells.

Nitric oxide has been described as a major messenger molecule regulating immune function, blood vessel dilatation and serving as a neurotransmitter in the brain and peripheral nervous system, (see review by Lowenstein and Snyder (1992)).

Three distinct forms of NO synthase have been described (Wood et al., 1994). Under basal conditions NO synthase activity in macrophages is negligible, while stimulation with lipopolysaccharide or gamma-interferon induces a massive enhancement of NO synthase. In blood vessels and neurons NO synthase is constitutive. NO is believed to act by binding to iron in the heme group at the active site of guanyl cyclase and to activate the enzyme to generate cGMP, which stimulates cGMP-dependent protein kinase (Ignarro, 1989; Lowenstein and Snyder, 1992). NO inhibits contraction of vascular smooth cells, and in addition NO has been identified as a major transmitter released from enteric inhibitory neurons. In a study of target cells for NO Young et al. (1993) showed high basal cGMP immunoreactivity in macrophages of the muscularis externa (Fig. 18a,c,c'). However, the level was not increased noticeably after exposure to sodium nitroprusside. Carbon monoxide (CO) like NO is an activator of guanyl cyclase, CO being formed by the action of the enzyme heme oxygenase. The neural localization of mRNA for the constitutive form of heme oxygenase has been demonstrated. This location is reported to be essentially the same as that of soluble guanyl cyclase (Verma et al., 1993). Since a potent selective inhibitor of heme oxygenase (zinc protoporphyrin-9) depletes endogenous

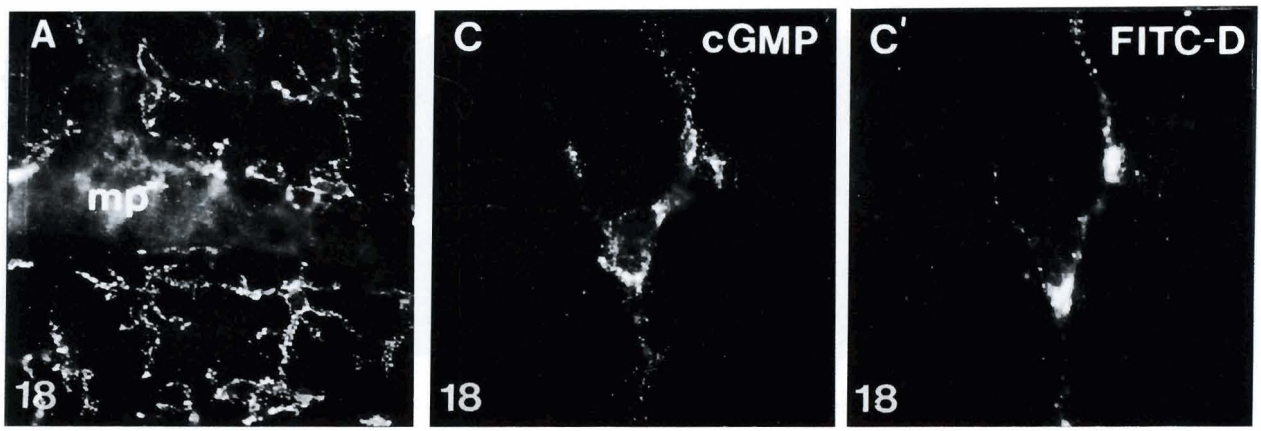


Fig. 18. A, C, C'. Guinea pig. **A.** Fluorescence micrograph of intestinal macrophages showing immunoreactivity for cGMP at the level of the myenteric plexus of ileum. mp: ganglion of the myenteric plexus. x 160. **C and C'** are paired micrographs showing co-localization of cGMP-IR (C) and FITC-dextran labelling (C') in serosal macrophages. x 400. From Young et al. 1993. Elsevier Science Ltd.

cGMP, Verma et al. (1993) suggest that CO like NO may be a physiologic regulator of cGMP, and suggest that CO may function as a neurotransmitter. Since cGMP has many functions the significance of its role in macrophages is unclear.

Acid phosphatase is described as an important enzyme in phagocytosis. However, macrophages in the human muscularis externa were mostly negative (Mikkelsen and Rumessen, 1992) as well as macrophages in the mouse (Mikkelsen, unpublished observation).

Collins et al. (1992) found evidence that an altered function of the enteric nervous system in nematode infected rats is mediated by interleukin-1 (a suppressive effect on acetylcholine release), and that the release of endogenous interleukin-1 most likely originates from macrophages. They suggest that if macrophages at Auerbach's plexus possess receptors for neuropeptides a neuroimmune axis would exist in the myenteric plexus.

Hartung et al. (1986) found specific binding sites on peritoneal macrophages for the neuropeptide substance P. Occupation of these sites on elicited macrophages mediated activation, i.e. activation of arachidonic acid metabolism, chemotaxis and oxidative bursts (Hartung and Tokya, 1983).

Other effects of substance P on macrophages/monocytes include production of interleukin-1, tumor necrosis factor-α, and interleukin-6 (Lotz et al., 1988; Laurenzi et al., 1990). These findings show that immunological and inflammatory responses could be regulated by nervous system-derived signals (Lotz et al., 1988). It is unclear if these conditions demonstrated for elicited macrophages also have a relevance for muscularis externa macrophages.

Since in bone marrow stroma macrophage processes make numerous contacts with neighbouring cells which display a high rate of proliferation and turnover, a growth regulation by the centrally located macrophages has been suggested (Crocker and Gordon, 1985). An interesting growth factor is the Steel factor also called the Kit ligand, stem cell factor or mast cell factor (Motro et al., 1991); it acts by binding to and activating its receptor, the c-Kit proto-oncogene product, which is a transmembrane protein tyrosine kinase. In W and Se mutant mice with malfunctioning genes at W and Steel loci developmental failure of three cell lineages: hemopoietic cells, melanocytes and germ cells has been demonstrated (Russell, 1979; Motro et al., 1991). By in situ hybridization RNA analysis Motro et al. (1991) showed that c-Kit protein and Steel factor are Kit receptor protein and Steel factor expressed in a wide variety of distinct anatomical sites in wild-type embryos.

Since it has been shown recently that the interstitial cells of Cajal express the c-Kit receptor protein (Huizinga et al. 1995), an attractive possibility is that the macrophages in close contact with interstitial cells of Cajal in a similar way could be involved in control of interstitial cells of Cajal via growth factors. With the c-Kit receptor expression on interstitial cells of Cajal it would appear that other cell types in the same location produce the ligand, the macrophages being attractive candidates.

In conclusion, the presently known physiology of the muscularis externa macrophages consists of scattered observations only. Comparing them with microglia and Langerhans cells, certain similarities are found. The cells are stellate ramified cells and appear to be endocytotically and secretory downregulated. Since microglia and Langerhans cells are also MHC class-II antigen positive and unactivated cells, it is intriguing if there is a common factor for muscularis externa macrophages, and Langerhans cells. They may be immunocompetent, participate in a neuroimmune axis, tissue growth and modulation, or other regulations of specific cell functions. Under normal circumstances they are inactivated and unlikely to be inflammatory, immunologically or neuroimmunologically active. Considering their ramified morphology, regular distribution and close spatial relationship to other cells, interaction with such cells by means of growth factors or other substances seems likely. Resident macrophages of the muscularis externa may become activated in Inflammatory bowel disease and thereby (neuroimmune axis) exert an influence on motility disturbances.

References

Anderson C.R. and Edwards S.L. (1993). Subunit b of cholera toxin labels interstitial cells of Cajal in the gut of rat and mouse. Histochemistry 100, 457-464.

Austyn J.M. and Gordon S. (1981). F4/80, a monoclonal antibody directed specifically against the mouse macrophage. Eur. J. Immunol. 11, 805-815.

Bennett A., Stamford I.F. and Stockely H.L. (1977). Estimation and characterization of prostaglandins in the human gastrointestinal tract. Br. J. Pharmacol. 61, 579-586.

Bennett A., Hensby C.N., Sanger G.J. and Stamford I.F. (1981). Metabolites of arachidonic acid formed by human gastrointestinal tissues and their actions on the muscle layers. Br. J. Pharmacol. 74, 435-444.

Botar J. (1966). The autonomic nervous system. An introduction to its physiological and pathological histology. Akadémiai Kiadó. Budapest. pp 1-442.

Cajal S.R. (1893). Sur les ganglions et plexus nerveux de l'intestin. C.R. Soc. Biol. 5, 217-223.

Cajal S.R. (1911). Histologie du système nerveux de l'homme & des vertébrés. Paris.

Chung L.P., Keshav S. and Gordon S. (1988). Cloning the human lysozyme cDNA: Inverted Alu repeat in the mRNA and in situ hybridization for macrophages and Paneth cells. Proc. Natl. Acad. Sci. USA 85, 6227-6231.

Coburn R.F. (1980). Evidence for oscillation of prostaglandin release from spontaneously contracting guinea pig taenia coli. Am. J. Physiol. 239, G53-G58.

Collins S.M., Hurst S.M., Main C., Stanley E., Khan I., Blennerhassett P. and Swain M. (1992). Effect of inflammation of enteric nerves. Cytokine-induced changes in neurotransmitter content and release. Ann. N.Y. Acad. Sci. 664, 415-424.

Cook R.D. and Burnstock G. (1976). The ultrastructure of Auerbach's plexus in the guinea-pig. II. Non-neuronal elements. J. Neurocytol. 5, 195-206.

Crocker P.R. and Gordon S. (1985). Isolation and characterization of resident stromal macrophages and hematopoietic cell clusters from mouse bone marrow. J. Exp. Med. 162, 993-1014.

Dijkstra C.D., Döpp E.A., Joling P. and Kraal G. (1985). The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 54, 589-599.

Dogiel A.S. (1899). Ueber den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Säugethiere. Arch. Anat. Entwicklungsgesch. 53, 130-158.

Faussone-Pellegrini M.S. (1985). Cytodifferentiation of the interstitial cells of Cajal related to the myenteric plexus of mouse intestinal muscle coat. An E.M. study from foetal to adult life. Anat. Embryol. 171, 163-169.

Faussone-Pellegrini M.S. (1987). Comparative study of interstitial cells of Cajal. Acta Anat. 130, 109-126.

Faussone-Pellegrini M.S., Pantalone D. and Cortesini C. (1990). Smooth muscle cells, interstitial cells of Cajal and myenteric plexus interrelationships in the human colon. Acta Anat. 139, 31-44.

Ferreira S.H., Herman A.G. and Vane J.R. (1976). Prostaglandin production by rabbit isolated jejunum and its relationship to the inherent tone of the preparation. Br. J. Pharmacol. 56, 469-477.

Feyrter F. (1951). Über die Pathologie der vegetativen nervösen Peripherie und ihrer ganglionären Regulationsstätten. Verlag Wilhelm Maudrich. Wien. pp 1-201.

Gabella G. (1979). Innervation of the gastrointestinal tract. Int. Rev. Cytol. 59, 129-193.

Golder J.P. and Doe W.F. (1983). Isolation and preliminary characterization of human intestinal macrophages. Gastroenterology 84, 795-802.

Gordon S., Todd J. and Cohn Z.A. (1974). In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. J. Exp. Med. 139, 1228-1248.

Gordon S., Perry V.H., Rabinowitz S., Chung L.P. and Rosen H. (1988). Plasma membrane receptors of the mononuclear phagocyte system. J. Cell Sci. Suppl. 9, 1-26.

Gordon S., Lawson L., Rabinowitz S., Crocker P.R., Morris L. and Perry V.H. (1992). Antigen markers of macrophage differentiation in murine tissues. In: Macrophage biology and activation (Current Topics in Microbiology and Immunology) Vol. 181. Gordon S. (ed). Springer. Berlin, Heidelberg. New York. pp 1-37.

Hartung H.P. and Tokya K.V. (1983). Activation of macrophages by substance P: induction of oxidative burst and thromboxane release. Eur. J. Pharmacol. 89, 301-305.

Hartung H.P., Wolters K. and Toyka K.V. (1986). Substance P: Binding properties and studies on cellular responses in guinea pig macrophages. J. Immunol. 136, 3856-3863.

Hogg N. (1987). Human mononuclear phagocyte molecules and the use of mononuclear antibodies in their detection. Clin. Exp. Immunol. 69, 687-694.

Huizinga J.D., Thuneberg L., Malysz J., Klüppel M., Mikkelsen H.B. and Bernstein A. (1995). W/Kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 373, 347-349.

Hume D.A. (1985). Immunohistochemical analysis of murine mononuclear phagocytes that express class II major histocompatibility antigens. Immunobiology 170, 381-389.

Hume D.A., Allan W., Hogan P.G. and Doe W.F. (1987). Immunohistochemical characterization of macrophages in human liver and gastrointestinal tract: Expression of CD4, HLA-DR, OKM1, and the mature macrophage marker 25F9 in normal and diseased tissue. J. Leukocyte Biol. 42, 474-484.

Ignarro L.J. (1989). Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. Circ. Res. 65, 1-21.

Keith A. (1915). A new theory of the causation of enterostasis. Lancet 2, 371-375.

Kelly P.M.A., Bliss E., Morton J.A., Burns J. and McGee J.O'D. (1988). Monoclonal antibody EBM/11: high cellular specificity for human macrophages. J. Clin. Pathol. 41, 510-515.

Keshav S., Chung P., Milon G. and Gordon S. (1991). Lysozyme is an inducible marker of macrophage activation in murine tissues as demonstrated by in sity hybridization. J. Exp. Med. 174, 1049-1058.

Kobayashi S., Furness J.B., Smith T.K. and Pompolo S. (1989). Histological identification of the interstitial cells of Cajal in the guinea pig small intestine. Arch. Histol. Cytol. 52, 267-286.

Kobayashi S. (1990). The structure of the autonomic end apparatus in the guinea pig small intestine and the problem of the interstitial cells of Cajal. Acta Med. Biol. 38, 103-127.

Komuro T. (1989). Three-dimensional observation of the fibroblast-like cells associated with the rat myenteric plexus, with special reference to the interstitial cells of Cajal. Cell Tissue Res. 255, 343-351.

Laurenzi M.A., Persson M.A.A., Dalsgaard C.-J. and Haegerstrand A. (1990). The neuropeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells are preferentially influenced by the neuropeptide. Scand. J. Immunol. 31, 529-533.

Lawrentjew B.J. (1926). Über die Verbreitung der nervösen Elemente (einschliesslich der «interstitiellen Zellen» Cajals) in der glatten Muskulatur, ihre Endigungsweise in den glatten Muskelzellen. Z. Mikrosk. Anat. Forsch. 6, 467-488.

Lawson L.J., Perry V.H., Dri P. and Gordon S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39, 151-170.

LeDuc L.E. and Needleman P. (1979). Regional localization of prostacyclin and thromboxane synthesis in dog stomach and intestinal tract. J. Pharmacol. Exp. Ther. 211, 181-188.

Lee S.H., Soyoola E., Chanmugam P., Hart S., Sun W.Q., Zhong H., Liou S., Simmons D. and Hwang D. (1992). Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. J. Biol. Chem. 267, 25934-25938.

LeFevre M.E., Hammer R. and Joel D.D. (1979). Macrophages of the mammalian small intestine: a review. J. Reticuloendothel. Soc. 26, 553-573.

Lotz M., Vaughan J.H. and Carson D.A. (1988). Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 241, 1218-1221.

Lowenstein C.J. and Snyder S.H. (1992). Nitric oxide, a novel biologic messenger. Cell 70, 705-707.

Mahida Y.R., Patel S., Gionchetti P., Vaux D. and Jewell D.P. (1989). Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum. Gut 30, 826-834.

Micklem K., Rigney E., Cordell J., Simmons D., Stross P., Turley H., Seed B. and Mason D. (1989). A human macrophage-associated antigen (CD68) detected by six different monoclonal antibodies. Br.

J. Haematol. 73, 6-11.

Mikkelsen H.B. and Rumessen J.J. (1992). Characterization of macrophage-like cells in the external layers of human small and large intestine. Cell Tissue Res. 270, 273-279.

Mikkelsen H.B., Thuneberg L., Rumessen J.J. and Thorball N. (1985). Macrophage-like cells in the muscularis externa of mouse small intestine. Anat. Rec. 213, 77-86.

Mikkelsen H.B., Mirsky R., Jessen K.R. and Thuneberg L. (1988a). Macrophage-like cells in muscularis externa of mouse small intestine: Immunohistochemical localization of F4/80, M1/70, and Ia-antigen. Cell Tissue Res. 252, 301-306.

Mikkelsen H.B., Thuneberg L. and Wittrup I.H. (1988b). Selective double staining of interstitial cells of Cajal and macrophage-like cells in small intestine by an improved supravital methylene blue technique combined with FITC-dextran uptake. Anat. Embryol. 178, 191-195.

Mikkelsen H.B., Rumessen I.T. and Thuneberg L. (1990). Prostaglandin H synthase immunoreactivity localized by immunoperoxidase technique (PAP) in the small intestine and kidney of rabbit and guinea-pig. Histochemistry 93, 363-367.

Mikkelsen H.B., Rumessen J.J. and Qvortrup K. (1991). Prostaglandin H synthase immunoreactivity in human gut. An immunohistochemical study. Histochemistry 96, 295-299.

Motro B., Van der Kooy D., Rossant J., Reith A. and Bernstein A. (1991). Contiguous patterns of c-kit and steel expression: analysis of mutations at the W and Se loci. Development 113, 1207-2121.

Naito M. (1993). Macrophage heterogeneity in development and differentiation. Arch. Histol. Cytol. 56, 331-351.

Nathan C.F. (1987). Secretory products of macrophages. J. Clin. Invest. 79, 319-326.

Nibbering P.H., Leijh P.C.J. and van Furth R. (1987). Quantitative immunocytochemical characterization of mononuclear phagocytes. II. Monocytes and tissue macrophages. Immunology 62, 171-176.

Ottaviani G. and Cavazzana P. (1940). Osservazioni sulle cellule interstiziali di Cajal. Arch. Ital. Anat. Embryol. 43, 75-89.

Ottaviani G., Castelli C.A. and Satta M. (1964). Osservazioni sopra le cellule interstiziali di Cajal. Acta Neuroveg. 26, 172-183.

Page R.C., Davies P. and Allison A.C. (1978). The macrophage as a secretory cell. Int. Rev. Cytol. 52, 119-157.

Pavli P., Hume D.A., Van de Pol E. and Doe W.F. (1993). Dendritic cells, the major antigen-presenting cells of the human colonic lamina propria. Immunology 78, 132-141.

Perry V.H. and Gordon S. (1989). Resident macrophages of the central nervous system: Modulation of phenotype in relation to a specialized microenvironment. In: Neuroimmune networks: Physiology and diseases. Goetzl E.J. and Spector N.H. (eds). Alan R. Liss, Inc. New York. pp 119-125.

Perry V.H. and Gordon S. (1991). Macrophages and the nervous system. Int. Rev. Cytol. 125, 203-244.

Rumessen J.J. and Thuneberg L. (1991). Interstitial cells of Cajal in human small intestine. Ultrastructural identification and organization between the main smooth muscle layers. Gastroenterology 100, 1417-1431.

Rumessen J.J., Thuneberg L. and Mikkelsen H.B. (1982). Plexus muscularis profundus and associated interstitial cells. II. Ultrastructural studies of mouse small intestine. Anat. Rec. 203, 129-146.

Rumessen J.J., Mikkelsen H.B. and Thuneberg L. (1992). Ultrastructure of interstitial cells of Cajal associated with deep muscular plexus of human small intestine. Gastroenterology 102, 56-68.

Rumessen J.J., Mikkelsen H.B., Qvortrup K. and Thuneberg L. (1993). Ultrastructure of interstitial cells of Cajal in circular muscle of human small intestine. Gastroenterology 104, 343-350.

Rumessen J.J. (1994). Identification of interstitial cell of Cajal. Significance for studies of human small intestine and colon. Dan. Med. Bull. 41, 275-293.

Russell E.S. (1979). Hereditary anemias of the mouse: a review for geneticists. Adv. Genet. 20, 357-460.

Sanders K.M. (1984). Role of prostaglandins in regulating gastric motility. Am. J. Physiol. 247, G117-G126.

Sanders K.M. and Ross G. (1978). Effects of endogenous prostaglandin E on intestinal motility. Am. J. Physiol. 234, E204-E208.

Sminia T. and Jeurissen S.H.M. (1986). The macrophage population of the gastrointestinal tract of the rat. Immunobiology 172, 72-80.

Sminia T. and van der Ende M.B. (1988). Localization, isolation and histochemical characterization of macrophages in the gut and mesenteric lymph nodes of the rat. Adv. Exp. Med. Biol. 237, 621-626.

Soesatyo M., Biewenga J., Kraal G. and Sminia T. (1990). The localization of macrophage subsets and dendritic cells in the gastrointestinal tract of the mouse with special reference to the presence of high endothelial venules. An immuno- and enzyme-histochemical study. Cell Tissue Res. 259, 587-593.

Taxi J. (1965). Contribution à l'étude des connexions des neurones moteurs du systeme nerveux autonome. Ann. Sci. Nat. Zool. 7, 413-674.

Taylor A.B., Kreulen D. and Prosser C.L. (1977). Electron microscopy of the connective tissues between longitudinal and circular muscle of small intestine of cat. Am. J. Anat. 150, 427-441.

Thuneberg L. (1982). Interstitial cells of Cajal: Intestinal pacemaker cells? Adv. Anat. Embryol. Cell Biol. 71, 1-130.

Thuneberg L. (1989). Interstitial cells of Cajal. In: Handbook of Physiology - The gastrointestinal system I. Schultz G.S. Wood J.D. and Rauner B.B. (eds). American Physiology Society. Bethesda, USA. pp 349-386.

Torihashi S., Gerthoffer W.T., Kobayashi S. and Sanders K.M. (1994). Identification and classification of interstitial cells in the canine proximal colon by ultrastructure and immunocytochemistry. Histochemistry 101, 169-183.

Vajda J. and Fehér E. (1980). Distribution and fine structure of the interstitial cells of Cajal. Acta Morphol. Acad. Sci. Hung. 28, 251-258.

van Furth R. (1989). Origin and turnover of monocytes and macrophages. In: Current topics in pathology 79. Cell kinetics of the inflammatory reaction. Iversen O.H. (ed). Springer. Heidelberg. 125-150.

van Rees E.P., Dijkstra C.D., van der Ende M.B., Janse E.M. and Sminia T. (1988). The ontogenetic development of macrophage subpopulations and Ia-positive non-lymphoid cells in gut-associated lymphoid tissue of the rat. Immunology 63, 79-85.

Verma A., Hirsch D.J., Glatt C.E., Ronnett G.V. and Snyder S.H. (1993). Carbon monoxide: a putative neural messenger. Science 259, 381-384.

Wilders M.M., Sminia T. and Janse E.M. (1983). Ontogeny of non-lymphoid and lymphoid cells in the rat gut with special reference to large mononuclear Ia-positive dendritic cells. Immunology 50, 303-314.

Winter H.S., Cole F.S., Huffer L.M., Davidson C.B., Katz A.J. and Edelson P.J. (1983). Isolation and characterization of resident

macrophages from guinea pig and human intestine. Gastroenterology 85, 358-363.

Wood P.L., Choksi S. and Bocchini V. (1994). Inducible microglial nitric oxide synthase: a large membrane pool. Neuroreport 5, 977-980.

Young H.M., McConalogue K., Furness J.B. and De Vente J. (1993). Nitric oxide targets in the guinea-pig intestine identified by induction of cyclic GMP immunoreactivity. Neuroscience 55, 583-596.

Zhou D.S. and Komuro T. (1992). Interstitial cells associated with the deep muscular plexus of the guinea-pig small intestine, with special reference to the interstitial cells of Cajal. Cell Tissue Res. 268, 205-216.