

# Polymyositis, Topological Proteomics Technology and Paradigm for Cell Invasion Dynamics

WALTER SCHUBERT<sup>a,b,\*</sup>

<sup>a</sup>Molecular Pattern Recognition Research Group, Institute of Medical Neurobiology, Otto-von-Guericke University of Magdeburg, Magdeburg, Germany; <sup>b</sup>MelTec Ltd, ZENIT Building, 39120 Magdeburg, Germany

(Received 1 August 2000; In final form 23 April 2001)

Polymyositis is an inflammatory myopathy characterized by muscle invasion of T-cells penetrating the basal lamina and displacing the plasma membrane of normal muscle fibers. This investigation presents a technology for the direct mapping of protein networks involved in T-cell invasion *in situ*. Simultaneous localization of 17 adhesive cell surface receptors reveals 18 different combinatorial expression patterns (CEP), which are unique for the T-cell invasion process in muscle tissue. Each invasion step can be assigned to specific CEP on the surface of individual T-cells. This indicates, that the T-cell invasion is enciphered combinatorially in the T-cells' adhesive cell surface proteome fraction. Given  $2^{17}$  possible combinations, the T-cell appears to have at its disposal a highly non-random restricted repertoire to specify migratory pathways at the cell surface. These higher-level order functions in the cellular proteome cannot be detected by large-scale protein profiling techniques from tissue homogenates. High-throughput whole cell mapping machines working on structurally intact tissues, as shown here, will allow to measure how cells of different origin (immune cells, tumor cells) combine cell surface receptors to encipher specificity and selectivity for interactions.

**Keywords:** Polymyositis; Proteomics; T-cells; Invasion; Imaging

## INTRODUCTION

There are two major types of immune cell infiltration in neuromuscular disorders: the polymyositis/inclusion body myositis (PM/IBM) and the dermatomyositis (DM) types. In the PM/IBM type of immune cell invasion, T-lymphocytes accumulating within the endomysium (space between muscle fibers) penetrate the basal lamina of intact muscle fibers (endomysial tube) and continuously displace and compress the muscle fiber plasma membrane—the sarcolemma. In the DM type of infiltration T- and B-lymphocytes accumulate in the perimysium and around blood vessels, but do not invade endomysial tubes. The invasion of muscle fibers by mononuclear cells in polymyositis has been extensively studied by immunofluorescence and electron microscopy (Arahata and Engel, 1984a,b; Engel and Arahata, 1984; Schubert *et al.*, 1993). It is a process, in which T-cells and macrophages, after penetration of the basal lamina of muscle fibers, send spike like projections into the muscle fiber surface. This leads to invasion, compression and finally replacement of

the muscle fiber by the mononuclear cells. During this process the sarcolemma membrane is not destroyed. It is displaced by the T-cells (Schubert *et al.*, 1993).

In the present investigation the PM/IBM type of mononuclear cell infiltration of the muscle tissue was investigated. Patients belonging to this diagnostic category may complain of myalgias and usually show chronic progressive symmetric weakness involving the muscles of the shoulder and the pelvic girdle. In muscle biopsies of PM patients the characteristic pathological feature implicated in the pathogenesis of the disease is represented by T-lymphocytes surrounding and invading normal muscle fibers (Engel and Arahata, 1984; Schubert *et al.*, 1993). The cause of the disease and the molecular mechanisms of the T-cell invasion are not known. A large number of investigations have shown that these muscle invasive T-lymphocytes express CD8 or CD4 cell surface antigens. As yet, there is no evidence for the immunologic nature of these T-cells although it has been suggested that antigen-specific cytotoxic events may take place (Engel and Arahata, 1984). However, an auto-antigen has not

\*Corresponding author. Address: Molecular Pattern Recognition Research Group, Institute of Medical Neurobiology, Otto-von-Guericke University of Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany. Tel.: +49-391-611-717-4. Fax: +49-391-611-717-6. E-mail: schubert@pc.mdlink.de

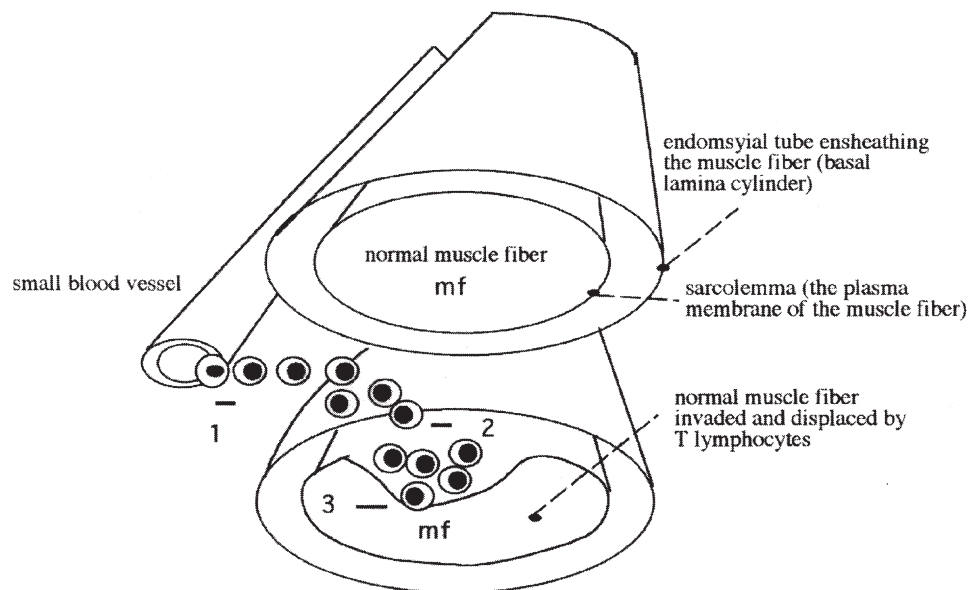


FIGURE 1 Schematic outline of T-lymphocyte (T-cell) invasion in polymyositis. 1: T-cells from the blood circulation enter the connective tissue of the skeletal muscle by migrating across the wall of a small blood vessel. 2: Starting from the connective tissue compartment between the muscle fibers (endomysium), the T-cells penetrate the basal lamina cylinder of an individual muscle fiber; after this penetration these T-cells are located between the basal lamina and the plasma membrane (sarcolemma) of the muscle fiber. 3: These front T-lymphocytes have a high migratory potential and are pace makers of a process of continuous displacement of the muscle fiber plasma membrane. This leads to increasing compression and damage of the muscle fiber.

been identified in polymyositis, and there is no clear morphological evidence for cytotoxic lyses of muscle fibers by T-cells, as it might be expected for an antigen-driven T-cell-to-muscle fiber interaction (Arahata and Engel, 1984b; Schubert *et al.*, 1993). Figure 1 gives a schematic presentation of a T-lymphocyte invasion process in polymyositis, and Fig. 2 shows the characteristic histologic features of an initial and a progressed stage of invasion.

Given that muscle-invasive T-cells (i) accumulate in the connective tissue between muscle fibers, (ii) then actively penetrate the endomyial tube of morphologically intact fibers, and (iii) progressively displace these fibers, these T-cells must have a high migration potential (Schubert *et al.*, 1993). The latter must involve differential adhesive mechanisms at the T-cell surface. These adhesive functions are likely to be different outside and inside the basal lamina cylinder surrounding muscle fibers, because the molecular components of these microenvironments are different: outside the endomyial tube, collagen and other extracellular matrix molecules characteristic for connective tissue are present, while inside the basal lamina cylinder (within the endomyial tube) the cell surface molecules of the muscle fiber sarcolemma are directly applied to the internal surface of the basal lamina.

In an attempt to identify candidate molecules for those adhesive functions, and, finally regulating T-cell invasion dynamics, a battery of proteins expressed at the T-cell surface was studied. Here I report on a new topological proteomic technology involving the simultaneous analysis of a multitude of combinatorial receptor patterns at the cell surface of invasive T-lymphocytes. The patterns, which

were found, provide clues for the disease specific organization of the T-lymphocyte infiltrate and the T-cell invasion process, based on differential adhesive mechanisms at the cell surfaces.

### SCREENING CANDIDATE T-CELL SURFACE MOLECULES BY MULTI-EPILOPE-IMAGING

As illustrated in Fig. 3, it is possible to simultaneously detect and selectively image a large number of different cell surface receptors in one and the same muscle tissue section by using sequential *in situ* antibody-epitope interaction imaging by means of fluorescence signals. The methodology has been evolved stepwise on the basis of earlier results (Schubert, 1992). Details of the methodology on the single cell level will be published elsewhere. The underlying method has allowed to examine different stages during the process of T-lymphocyte invasion of endomyial tubes at the level of combinatorial CD-antigen patterns expressed at the cell surface of muscle invasive T-cells.

Figure 3 gives an example of the simultaneous detection of 17 cellular protein markers, which are characteristic for the muscle invasion process. Simultaneous mapping allowed to distinguish between invasive T-lymphocytes, cross sectioned muscle fibers and small blood vessels within the muscle tissue. The example shows an initial process of T-lymphocyte invasion, in which several CD4 positive and CD8 positive T-lymphocytes have penetrated a small blood vessel and are now localized within the endomyial connective tissue between muscle fibers.

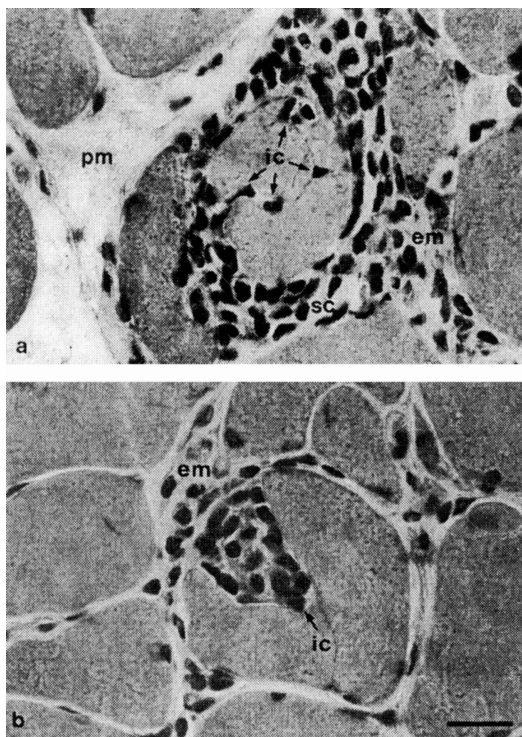


FIGURE 2 Histologic features of polymyositis. Transmitted light micrographs of muscle cross sections (stained with hematoxylin eosin) illustrating invasion/displacement of morphologically normal muscle fibers by mononuclear T-cells. One initial (a) and one advanced stage (b) of this process are shown. Displacement is initiated by mononuclear cells surrounding the muscle fiber (a, surrounding cell: sc): only few pacemaker cells invade the endomysial tube that is occupied by the intact muscle fiber (a, invasive cells: ic). Absence of mitoses indicates that these cells do not proliferate. Progression of this process is characterized by an army of additional invasive cells (b, ic), which compress and finally replace the fiber. Note the normal morphology of invaded fibers as compared to adjacent non-invaded fibers. Em, endomysium; Pm, perimysium; bar: 22  $\mu$ m.

Interestingly this simultaneous analysis indicates, that the initially invasive T-cell (the front T-lymphocyte) (Fig. 3h, arrow), which penetrates the basal lamina cylinder of a morphologically intact muscle fiber, down-regulates a

large number of cell surface proteins which are frequently expressed by T-cells accumulating behind the invasive front, outside the endomysial tube (Fig. 3i-p). The invasive front T-lymphocytes may be CD8 positive or CD4 positive cells (Arahata and Engel, 1984b; Schubert *et al.*, 1993). They displace muscle fibers expressing the neural cell adhesion molecule (NCAM) (Schubert *et al.*, 1989; 1993; Mundegar *et al.*, 1995).

The proteins detected simultaneously in Fig. 3 are specified in Table I. A detailed analysis of the example given in Fig. 3 is documented in Table II giving the characteristic phenotypes obtained by overlay of each fluorescent protein signal shown in Fig. 3. Figure 4 gives a higher magnification of the invasion site, and Fig. 5 illustrates by color overlay that the CD8 positive T-cell indicated by an arrow in Fig. 3h and Fig. 4a, b (arrow) indeed is located between the basal lamina and the muscle fiber surface: it is the initially invasive "pacemaker" T-cell.

#### THE ALZHEIMER AMYLOID PRECURSOR PROTEIN (APP) IS A MARKER FOR INVASIVE FRONT T-LYMPHOCYTES

APP is a molecule, which plays an important part in the pathogenesis of Alzheimer's disease (Kang *et al.*, 1987; Tanzi *et al.*, 1987; Kitaguchi *et al.*, 1988; Schubert *et al.*, 1991). The molecule is also expressed in a variety of cell types including neurons and cells of the immune system (Schubert *et al.*, 1991; 1993). When muscle tissue sections showing abundant T-cell infiltrations are examined with antibodies against the N-terminal domain of APP (i.e. monoclonal antibody 22c11) it is seen that approximately one third of all T-cells are APP positive (Schubert *et al.*, 1993). These APP<sup>+</sup> T-cells are located at the invasive site, consistently show the highest level of APP expression and are either CD8<sup>-</sup> CD4<sup>+</sup>, CD8<sup>+</sup> CD4<sup>-</sup>

TABLE I Specification of proteins and carbohydrate structures localized simultaneously by an antibody and lectin library

Protein (p), lectin (l)	Specification
CD2 (p)	SRBC receptor, ligand for LFA-3
CD3 (p)	CD3 complex associated with T-cell antigen receptor TCR
CD4 (p)	Co-recognition receptor for MHC class II with TCR
CD7 (p)	Fc receptor for IgM "Fc $\mu$ R"
CD8 (p)	Co-recognition receptor for MHC class I with TCR
CD11b (p)	$\alpha$ M integrin chain of MAC-1 complex
CD 16 (p)	Fc $\gamma$ RIII receptor for selective binding of IgG 1 and IgG3
CD36 (p)	GP IV, collagen receptor
CD38 (p)	gp 45 receptor involved in leucocyte activation
CD45RA (p)	Restricted leucocyte common antigen isoform containing at least exon A
CD56 (p)	NCAM
CD57 (p)	IINK-1 receptor
CD62L (p)	L-selectin
CD71 (p)	Transferrin receptor
HLA-DR (p)	MHC class II receptor
HLA-DQ (p)	MHC class II receptor
APP (p)	Alzheimer amyloid precursor protein
UEAI (l)	Ulex-europaeus I agglutinin recognizing $\alpha$ -L-fucose groups in carbohydrate structures
Laminin A (p)	Basal lamina protein

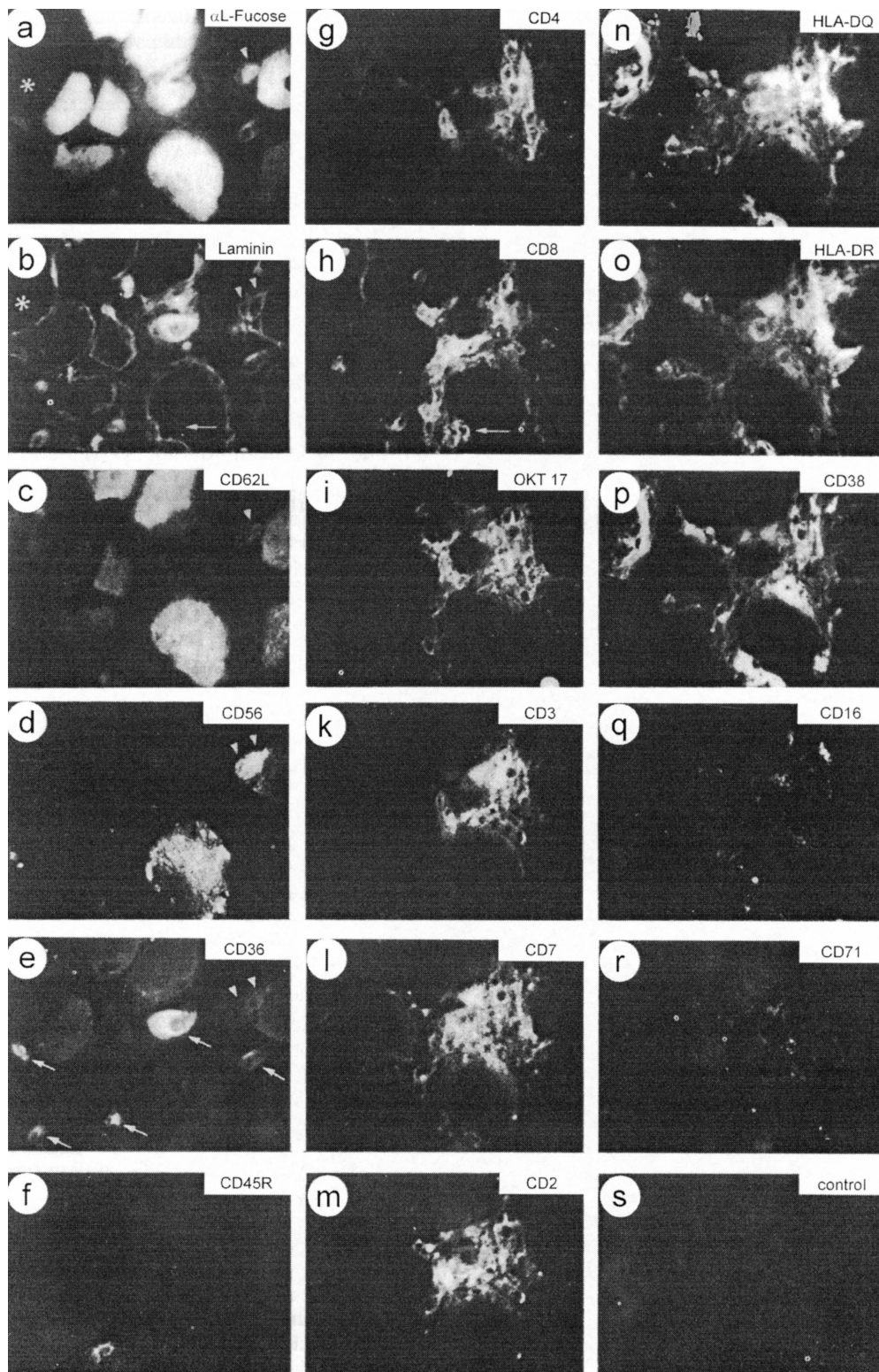


FIGURE 3 Simultaneous localization of cellular differentiation molecules with a 17-mer antibody-ligand library in a muscle tissue section of a patient with polymyositis. Sixteen antibodies recognize epitopes expressed by adhesive proteins (b)–(r), one lectin ligand recognizes  $\alpha$ -L-fucose carbohydrate groups (a). Note that in (a)–(f) differentiation molecules associated with endothelial cells or with muscle fibers and developing muscle cells during regeneration are seen. In (g)–(r) cell surface adhesive proteins associated with invasive T-lymphocytes are shown. (s) indicates negative signal of an irrelevant antibody (non-immune IgG). Each cell is characterized by a unique combinatorial molecular expression pattern, which is summarized in Table II, showing the result of an extended analysis of 16 tissue samples. Bar: 60  $\mu$ m.

TABLE II Binary codes of cell surface proteome fractions, which are characteristic for T-cell invasion in polymyositis, and identification of additional proteome fractions specific for non-T-cells in muscle tissue\*

proteins / glyco-structures	APP	CD 16	CD 71	CD 11b	CD 38	HLA DR	HLA DQ	CD 2	CD 7	CD 3	CD 8	CD 4	CD 45 RA	CD 36	CD 56	CD 62 L	CD 57	Lamini-A	lectin	UEA I
pv	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
pv	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
em	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pre-in	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
in	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
mature muscle fibres	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1
developing myotubes (regeneration)	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1
endothelial cells	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0

fraction 1
  fraction 2
  fraction 3
  fraction 4
  fraction 5
  fraction 6
  fraction 7
  fraction 8

\*The proteins are specified in Table I. The present binary codes were obtained by multi-epitope-imaging. pv, perivascular accumulation of T-cells; em, migration of T-cells across the connective tissue of the endomysium; pre-in, T-cells contacting the basal lamina; in, penetration of pace maker T-cell across the basal lamina cylinder, thereby invading the endomysial tube of the muscle fiber. Note that the arrow denotes the invasion pathway of T-cells entering the muscle tissue from the blood circulation.

T-cell phenotypes, or CD8<sup>+</sup> CD4<sup>+</sup> T-cells chimeras. In contrast, a paucity of all endomysial T-lymphocytes show APP expression, both in PM and miscellaneous neuromuscular disorders. A comparison of all invading and endomysial T-cells revealed that the most significant increase of invading APP<sup>+</sup> T-cells is found within the CD8<sup>-</sup> CD4<sup>+</sup> subset. None of the other APP<sup>+</sup> T-cell subsets is increased at statistical significance (Schubert *et al.*, 1993).

The APP<sup>+</sup> non-T-cells are preferentially located in the endomysium outside the endomysial tube (basal laminar cylinder). Parts of the latter cells are endothelial cells. We have also found a significant accumulation of the CD8<sup>+</sup> CD4<sup>-</sup> APP<sup>-</sup> T-cell subset among the invading cells inside the endomysial tube. However, these cells are mainly accumulated behind the invasive front. It is interesting to note, that the majority of the CD8<sup>+</sup> CD4<sup>+</sup> T-cells (T-cell chimeras),

representing only a minority of the mononuclear cell population in muscle tissue, express APP (1–5%).

Figure 6 gives an example showing an initially invasive front T-lymphocyte, that has penetrated the basal lamina cylinder of a cross sectioned muscle fiber. This T-cell expresses the CD8<sup>+</sup> phenotype and additionally expresses the Alzheimer Amyloid Precursor Protein (APP), that is highly concentrated at the leading edge of a cell extension of this T-cell interdigitating with the muscle fiber surface. The latter is highly positively stained for NCAM (Schubert *et al.*, 1993).

Figure 7 shows a more progressed stage of T-lymphocyte invasion of the endomysial tube, indicating that APP expression is totally restricted to T-cells located at the leading front, whereas T-cells behind the invading front are negative for APP.

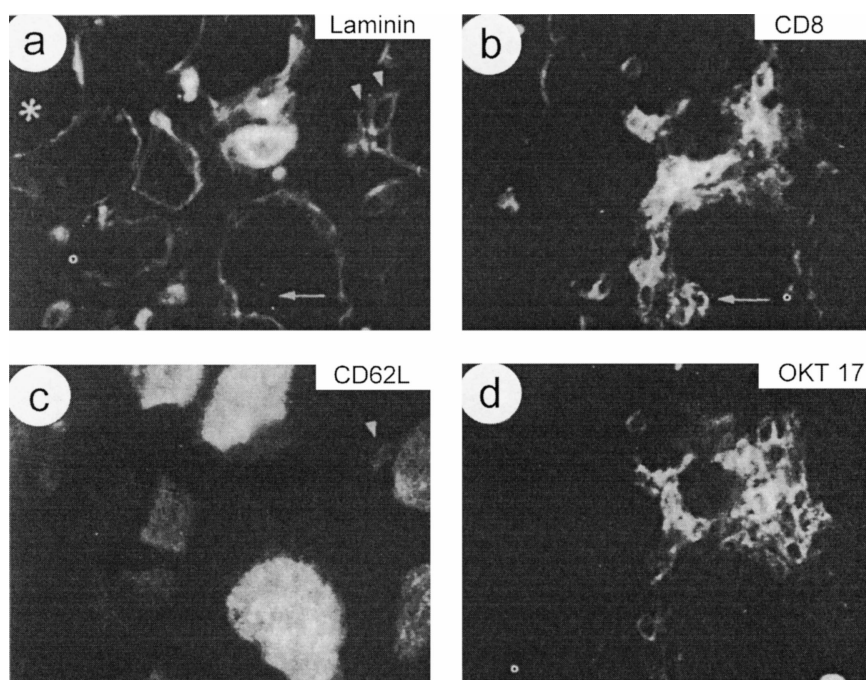


FIGURE 4 Illustration of a sectional view of the signals shown in Fig. 3b, c, h, i. The arrow in (a) indicates that the position of a pacemaker T-cell (b, arrow), which has penetrated the basal lamina cylinder, and is located between the basal lamina and the muscle fiber (c). Note that this T-cell does not express the OKT 17 protein, which is, however, expressed by CD8 T-cells outside the endomysial tube. Bar: 100  $\mu\text{m}$ .

### SYNOPSIS OF APP EXPRESSION DURING T-CELL INVASION

It has been shown, that all three major splice products of APP ( $\text{APP}_{695}$ ;  $\text{APP}_{751}$ ;  $\text{APP}_{770}$ ) are synthesized and actively secreted by human peripheral blood lymphocytes (PMBL) following stimulations with several mitogens (Mönning *et al.*, 1990). The time course of induction was similar to that of interleukin 2 and interleukin 2 receptor, and the APP-isoform predominantly secreted by T-cells was  $\text{APP}_{751}$  containing the Kunitz-type proteinase-inhibitor-domain. In contrast to stimulated PMBL, T-cells invading muscle fibers do not show co-expression of APP and interleukin 2 receptors (Schubert *et al.*, 1993). Expression of transferrin receptors, which are early markers of proliferating T-lymphocytes, as well as T-cell secretion of IL 2 (Isenberg *et al.*, 1986) is not observed in PM. Mitoses of T-cells are also absent (Schubert *et al.*, 1993). Thus  $\text{APP}^+$  T-cells invading muscle fibers are non-proliferating lymphocytes with cell surface properties that are different from antigen- or mitogen-stimulated PMBL *in vitro*. In addition, invasive T-cells simply displace but not destroy sarcolemma membranes in PM (Arahata and Engel, 1984b). Together these findings are not well compatible with a cytotoxic action of the invasive T-cells. On the basis of these earlier results and the present findings it is clear that early response gene products, which are usually co-expressed during T-cell activation (IL 2, IL 2 receptor, transferrin receptor, etc.), are not found in muscle-invasive T-cells.

The present data together indicate that APP must have a dominant role in the T-cell invasion of muscle. Since there is a strict compartmentalization of the APP molecules within T-cell infiltrates, it is suggested that the role of APP is to drive T-cell migration inside the endomysial tube: T-cell surface associated APP, which is highly expressed in the T-cell penetrating the basal lamina cylinder, may mediate the interaction of the front T-lymphocytes with the muscle fiber surface. Interestingly these  $\text{APP}^+$   $\text{CD8}^+$  or  $\text{APP}^+$   $\text{CD4}^+$  pacemaker T-cells are negative for a battery of 15 other cell surface molecules, including the CD3 signal transducing complex clearly indicating that these cells are not involved in cell activation processes (Table II).

### T-CELL INVASION DYNAMICS

#### Technical Remarks Related to Direct Detection of Protein Networks *In Situ*

Mapping large protein networks within a cell or at the cell surface is dependent on repetitive incubation-imaging bleaching cycles using large fluorochrome-conjugated antibody libraries (Schubert, 1998). Given large antibody libraries ( $>$  tens or hundreds of monoclonal antibodies) it can be observed and measured in detail that specificity of the antibody-epitope interactions within each subvolume of a cell (i.e. voxel) is qualitatively and quantitatively reproducible, independent of the sequential position of a given antibody in a library. This is obviously due to the

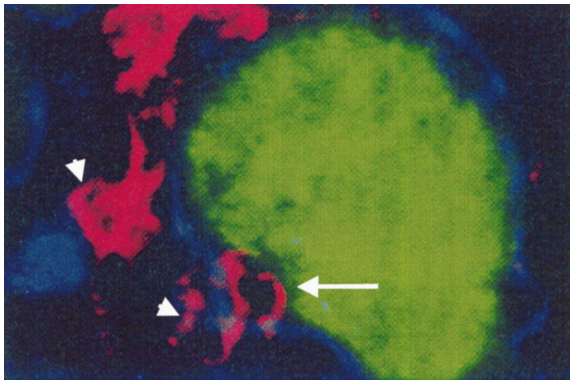


FIGURE 5 Colored overlay of the signals shown in Fig. 4a–c. The arrow indicates that the initially invasive pacemaker T-cell (red) is located between the muscle fiber expressing the L-selectin epitope Leu8 (green) and the basal lamina-associated protein laminin A (blue). Other CD8 T-cells are located in the endomysium outside the basal lamina cylinder (arrows).

protein organization principles in a cell allowing macromolecules to diffuse by a kinetics like in water and to specifically interact with the target molecule, despite the overall high protein concentration within the corresponding cell's compartment (details will be published elsewhere). This has allowed to map, cell by cell, within the muscle tissue a 17-mer-antibody library recognizing cell surface adhesive receptors. This library allowed to identify the protein compositions, which are characteristic for the T-cell invasion process. The library was selected out of 100 other antibodies against different adhesive cell surface proteins within a couple of years. The selected antibodies were found to recognize adhesive molecules, which together express specific cell patterns in defined functional contexts.

### Topological Identification of Proteome Fractions Involved in T-cell Invasion

By analyses using multi-epitope-imaging, cell by cell (to be published in detail elsewhere), 18 different combinatorial cell surface patterns were found to be

highly characteristic for the T-cell invasion process in polymyositis. These patterns are listed as binary codes in Table II. Another nine combinatorial patterns were found to be characteristic for muscle cells and endothelial cells present in the same tissue.

Following this analysis, the extravasation of T-lymphocytes in muscle tissue (penetration of small blood vessels and perivascular accumulation) selects T-cells that differentially combine five different out of the 17 measured cell surface molecules, while avoiding the expression of the remaining 12. The latter patterns are termed “fraction 1” of the adhesive cell surface proteome, because it can be assigned to clear-cut and topologically defined functions related to the perivascular region (see Table II, fraction 1). Migration of T-cells across the endomysium is characterized by usage of fraction 2, which comprises a combination of CD8, HLADR and HLADQ molecules combined with molecules of fraction 1 omitting CD4. This migration period is dominated by CD8-expressing T-cells. The more the T-cell migration approaches towards an individual muscle fiber, which is ensheathed by the basal lamina cylinder, the more the T-cells express a “close to invasion phenotype”. The latter is characterized by fraction 3 (see Table II) strictly avoiding the expression of CD2. Finally, T-cells, which directly face the basal lamina of a muscle fiber, express fraction 4, which is a clear-cut minus variant of fraction 3 by highly restricted differential expression of CD8, CD3 and CD7, which obviously precedes the T-cell penetration of the basal lamina cylinder. T-cells that have penetrated the latter (pacemaker T-cells) show the most restricted phenotype defined by a combination of CD8 and APP, whilst all remaining 15 receptors are not expressed (invasion-fraction 5).

Together these data imply, that T-cells migrating from the perivascular region to the site of invasion of the basal lamina cylinder permanently change the composition of adhesive proteins at their cell surface. Several lines of evidence indicate this.

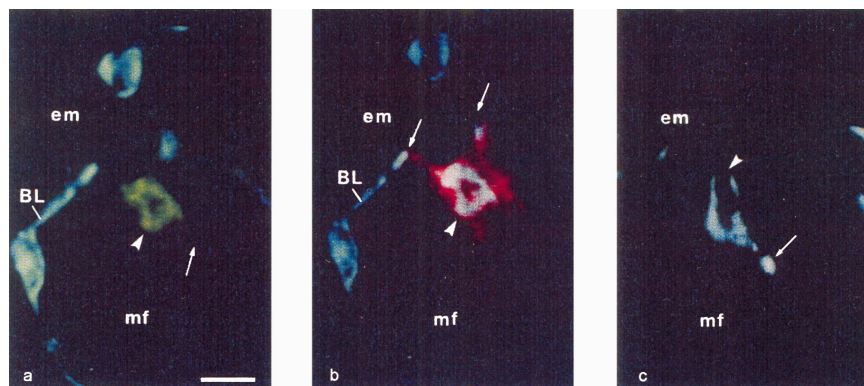


FIGURE 6 Initial stage of muscle fiber displacement in polymyositis mediated by an APP<sup>+</sup> CD8<sup>+</sup> T-lymphocyte. Four colors illustrate the direct correlation of CD8 (green), APP (white), laminin A (blue), and NCAM (CD56) (red). Note that this T-lymphocyte has penetrated the basal lamina and that APP is concentrated on the tip of a T-cell extension displacing the NCAM expressing muscle fiber (c, optical section across (b)). Bar: 10  $\mu$ m.

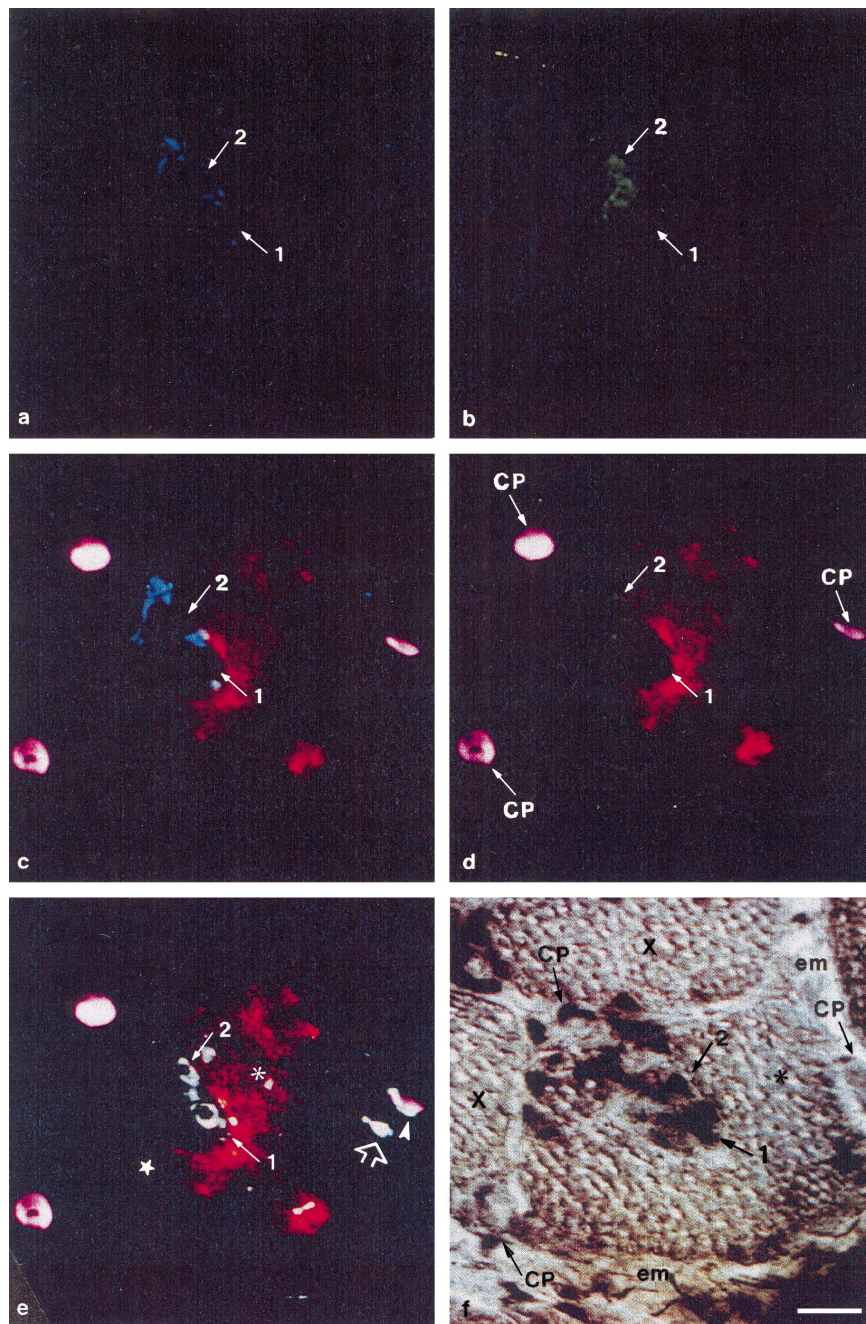


FIGURE 7 Progressed stage of muscle fiber invasion by T-cells. (blue) CD8; (green) CD 4; (red) NCAM (CD56); (white) APP; (purple) UEAI lectin (endothelium of capillaries). Note that several T-cells have progressively displaced a muscle fiber expressing high levels of NCAM (CD56, red). Only the invasive front T-cells express APP (white), whilst the cells behind the invasive front do not express APP. APP<sup>+</sup> T-cells co-express CD4 (arrow 2) or CD8 (arrow 1). In (f) the normal morphologic structure of the tissue is illustrated simultaneously by staining the tissue section with hematoxylin eosin. CP: capillaries. Em, endomysium; \*, muscle fiber structure; X, normal non-invaded muscle fibers; bar: 20  $\mu$ m.

First, cell surface protein patterns which are observed either in proteome fraction 4 or 3 (Table II) are never observed in the perivascular region (fraction 1).

Second, there is no redundancy of cell surface protein patterns during the process of invasion (from the perivascular area to the muscle fiber area).

Third, fraction 5 (Table II), which is unique to T-cells penetrating the basal lamina cylinder, is never observed in T-cells in the perivascular area or in the endomysium between the muscle fibers. The latter fraction showing a

high restriction of protein combinations to only two adhesive receptors, also indicates, that T-cells penetrating the basal lamina cylinder should be incapable of signaling and activation via the TCR, because the corresponding receptors are not expressed (CD3 and CD2). Absence of the obligatory CD3 zeta chain can be also shown by RT-PCR analysis (in preparation).

In polymyositis the muscle-invasive T-cells adhere to each other as a dense lymphoid network (see Fig. 1), and on the other hand must exert an overall highly migratory



potential directed against a given muscle fiber. It is therefore inferred that the observed proteome fractions 1–5 are involved in T-cell- to T-cell adhesion, and together act as an intercellular network producing an adhesive gradient, from the perivascular area to the site of T-cell penetration. This may exert a push and pull mechanism moving the T-cells towards the muscle fiber.

This conclusion is supported by the fact that T-cells surrounding blood vessels and migrating across the endomysium quantitatively and qualitatively combine more adhesive proteins than T-cells penetrating the basal lamina of a muscle fiber.

Together it is shown that T-cells in muscle tissue express selectivity for muscle invasion by differentially combining 9 out of 17 adhesive cell surface proteins. Given all theoretically possible combinations of 17 adhesive proteins, the T-cell has at its disposal a  $2^{17}$ -dimensional data space. Within that data space, the T-cell “occupies” only 18 defined cluster areas, thereby defining a limited T-cell repertoire for the muscle tissue compartment. These clusters differ substantially from those seen in T-cells in the blood (in preparation). Interestingly, muscle cells use the same  $2^{17}$ -dimensional data space different stages of development and also by endothelial cells (see Table II, fractions 6–8). The latter cell types however “occupy” cluster areas, that are inherently different from those occupied by the T-cells. This indicates that different cell types are specifically characterized by highly restricted combinatorial proteome fractions.

On the basis of these data it is indicated that a cell's proteome can be subdivided into fractions, which are specific and selective for each cell in a tissue. The large scale reading of those proteome fractions in the context of tissues will provide us with combinatorial information encoding cellular functions.

## DISCUSSION

In the present paper a new technology enabling the simultaneous direct localization of a large number of different cell surface adhesive proteins was applied to map the T-cell invasion process in different stages of invasion. Since muscle- invasive T-cells first accumulate around small blood vessels, then migrate across the connective tissue between the muscle fibers, and then penetrate the basal lamina cylinder of given muscle fibers, both the origin and the direction of the T-cell invasion are known. Hence, the different stages of T-cell invasion can be clearly identified in muscle biopsy tissue from polymyositis patients. The multi-epitope imaging technology was focused on many different stages of T-cell invasion in order to map the combination patterns of adhesive proteins. When each single adhesive protein is detected by a fluorescence signal, and is registered as present or absent per cell (1/0), a combinatorial binary map of the

analyzed monoclonal antibody library is obtained (see Table II).

According to these binary data, the process of T-cell invasion in polymyositis can be described as a highly non-random dynamic event, in which migratory T-cells use a restricted repertoire of combinations of adhesive cell surface proteins. Interestingly, there are only 18 different combinatorial patterns that are used in a temporally and spatially defined manner by the muscle-invasive T-cells, whilst there are  $2^{17}$  different combinations at a theoretical disposal. This is important to note, because it may suggest, that T-cells invading any site within an organism specify this invasion by usage or other combinatorial programs represented in their “data space”. For example, there appear to be combinations of adhesive cell surface proteins that are unique for T-cells in the blood (manuscript in preparation). This strongly suggests that T-cells can activate compartment-specific gene programs for the expression of appropriate protein compositions at their cell surface.

Together these data indicate that there is a higher level organization in the cellular proteome that can be detected when large proteome fractions are simultaneously localized in cells and tissues. Consequently it is important to recognize that the proteome of each given cell encodes cellular functions by differentially combining the proteins in the cell's compartments. To obtain this three dimensionally enciphered information from different types of cells, tissues, and disease states, requires the use of high-throughput whole cell mapping machines, which will become an important issue in future proteomics research.

## Acknowledgements

The technical assistance of M. Bode, M. Friedenberger, F. Wedler, in preparing the manuscript is greatly acknowledged. The work was supported by DFG through SFB 387, Schu 627/8-2, and BMBF 01ZZ9510/07NBL04.

## References

- Arahata, K. and Engel, A.G. (1984a) “Monoclonal antibody of mononuclear cells in myopathies I. Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T-cells”, *Ann. Neurol.* **16**, 193–208.
- Arahata, K. and Engel, A.G. (1984b) “Monoclonal antibody analysis of mononuclear cells in myopathies. III. Immunoelectron microscopy aspects of cell-mediated muscle fiber injury”, *Ann. Neurol.* **19**, 112–125.
- Engel, A.G. and Arahata, K. (1984) “Monoclonal antibody analysis of mononuclear cells in myopathies. II. Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis”, *Ann. Neurol.* **16**, 209–215.
- Isenberg, D.A., Rowe, D., Shearer, M., Novic, D. and Beverley, P.C.L. (1986) “Localization of interferons and interleukin 2 in polymyositis and muscular dystrophy”, *Clin. Exp. Immunol.* **63**, 450–458.
- Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987) “The precursor of Alzheimer's disease amyloid A4 protein resembles a cell surface receptor”, *Nature* **325**, 733–736.

- Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. and Itoh, H. (1988) "Novel precursor of Alzheimer's disease amyloid precursor shows protease inhibitor activity", *Nature* **331**, 530–532.
- Mönning, U., König, G., Prior, R., Mechler, I.I., Schreiter-Gasser, U., Masters, C.L. and Beyreuther, K. (1990) "Synthesis and secretion of Alzheimer  $\beta$  precursor protein by stimulated human peripheral blood leukocytes", *FEBS Lett.* **277**, 261–266.
- Mundegar, R.R., von Oertzen, J. and Zierz, S. (1995) "Increased laminin A expression in regenerating myofibers in neuromuscular disorders", *Muscle Nerve* **18**, 992–999.
- Schubert, W. (1998) "Molecular semiotic structures in the cellular immune system: key to dynamics and spatial patterning?", In: Parisi, J., Müller, S.C. and Zimmermann, W., eds, *A Perspective Look at Non-linear Physics Lecture Notes in Physics*, (Springer, Berlin) **Vol. 503**, pp 197–206.
- Schubert, W., Zimmermann, K., Cramer, M. and Starzinski-Powitz, A. (1989) "Lymphocyte antigen Leu19 as a molecular marker of regeneration in human skeletal muscle", *Proc. Natl. Acad. Sci. USA* **86**, 307–311.
- Schubert, W., Prior, R., Weidemann, A., Dirksen, I.I., Mulhaupt, G., Masters, C.L. and Beyreuther, K. (1991) "Localization of Alzheimer  $\beta$ A4 precursor protein at central and peripheral synaptic sites", *Brain Res.* **563**, 184–194.
- Schubert, W., Masters, C.L. and Beyreuther, K. (1993) "APP + T-lymphocytes selectively sorted to endomysial tubes in polymyositis displace NCAM-expressing muscle fibers", *Eur. J. Cell Biol.* **62**, 333–342.
- Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A.P., St George-Hyslop, P., Van Keuren, M.L., Patterson, D., Pagan, S., Kurnit, D.M. and Neve, R.L. (1987) "Amyloid  $\beta$  protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus", *Science* **235**, 880–883.