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Review

L is for lytic granules: lysosomes that kill

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1. Introduction

Lysosomes are found in all eukaryotic cell types, and function as organelles specialised in protein degradation. A series of hydrolases packaged within the lysosomes are able to degrade proteins delivered to this acidic compartment. Proteins can be delivered to the lysosome via both biosynthetic and endocytic routes. In addition, lysosomes are able to engulf other intracellular organelles by a process called autophagy.

Proteins delivered to lysosomes are rapidly degraded, and these organelles form an essential part in maintaining proper function in all cell types. In the majority of cells the lysosome functions only as an intracellular organelle. However, in cytotoxic T lymphocytes (CTL) and a small number of other cell types, the lysosome is also a secretory organelle that contains specialised secretory proteins in addition to the lysosomal hydrolases. In the case of CTL, the secretory proteins are specialised for the destruction of other cells: so that by secreting it's lysosomal contents the CTL is now able to kill other cells. These specialised lysosomes of the CTL are known as lytic granules. They are dual functional organelles which can not only degrade proteins targeted to them within the cell, but can also destroy whole cells as a consequence of their secretion. This article reviews what is known about the specialised lysosomes of CTL and the mechanisms that are required to modify an intracellular lysosome into a destructive secretory granule.

2. CTL function and biochemistry

2.1. The function of CTL

CTL represent the body's primary defence against viral-infected and tumourigenic cells and are involved in autoimmune disorders and transplant rejection. These functions are possible because of the ability of CTL to recognise other cells as ''foreign'' and destroy them. Most cells within the body present fragments of proteins, generated within the cell, on the cell surface in the context of the Major Histocompatibility Complex (MHC) proteins (reviewed [1]). The MHC proteins are highly polymorphic enabling CTL to distinguish between self and non-self MHC. The MHC-peptide complex serves as an indicator of both cell type (self or foreign) and cell status (''normal'' or transformed/infected). Thus, CTL patrol the body to check for foreign cells, by engaging MHC-peptide complexes with the T cell receptor (TcR). Normal self cells are ''ignored'' during this process - they do not activate the CTL and therefore are allowed to live. However, as a result of either transformation or viral infection, non-self proteins are presented in conjunction with a cell's MHC proteins, and this cell is subsequently recognised as foreign by the CTL. Once activated, the CTL is able to initiate a series of events which culminate in death of the recognised cell. Should a CTL be able to recognise a self protein-MHC complex, autoimmune disorders may ensue, in which CTL destroy normal self cells. Alternatively, if cells presenting non-self-MHC proteins are introduced to the body, for example during organ transplantation, these cells can also be recognised as foreign and destroyed.

2.2. Mechanisms of killing

Following recognition of a target cell through the TcR, a number of signals, including protein tyrosine phosphorylation and elevation of intracellular calcium levels (reviewed [2]), are generated within the CTL. These signals culminate in the induction of transcription of the effector proteins and, after several days, the appearance of electron-dense granules within the CTL. The effector proteins are the mediators of CTL function and are stored within the granules until required. Subsequent interaction with a target cell results in polarisation of the Golgi apparatus and the microtubule organising centres of the CTL towards the point of contact with the target cell [3–6], and directed exocytosis of the granules towards the target cell. During granule-mediated killing, the cytolytic proteins released from the granules inflict a ''lethal hit'' on the target cell and the cell dies (Fig. 1).

Alternatively, CTL may utilise a cell surface receptor to induce target cell death. A number of cell types express a surface protein called Fas/APO-1/CD95 which contains a ''death domain'' in its

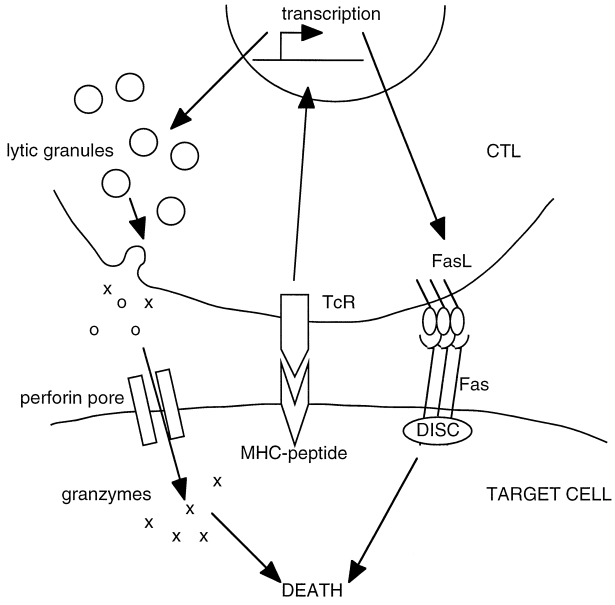

Fig. 1. Mechanisms of CTL-mediated cytotoxicity. At least two cytolytic mechanisms are used by CTLs to destroy target cells. Recognition of a foreign MHC-peptide complex through the T cell receptor (TcR) initiates signals within the CTL that result in transcriptional activation of function-related genes. Perforin and the granzymes are stored in lytic granules within the CTL. Upon further stimulation of the TcR, lytic granules are exocytosed towards the target cell. Perforin forms a pore in the target cell membrane, allowing the granzymes to enter the target cell and induce death. During Fas-mediated cytotoxicity, TcR stimulation results in upregulated FasL expression on CTL surfaces. FasL cross-links Fas on the target cell. A death-inducing signalling complex (DISC) is recruited to the cytoplasmic tail of Fas, resulting in transduction of a death signal to the target cell.

cytoplasmic region [7]. During CTL activation, levels of a protein named Fas-ligand (FasL) are upregulated on the CTL surface. FasL can cross-link target cell Fas receptors, and the oligomerised receptors can then transduce a death signal to the target cell through a number of associated proteins [8,9]. Following the initial interaction, which results in FasL expression, no further specific recognition of the target cell through TcR is required for Fas-mediated cytotoxicity. Thus, a FasL bearing CTL is able to destroy any Fas-positive cell, whether it has been specifically recognised or not. The two mechanisms used by CTL to destroy target cells are summarised in Fig. 1.

While the granule-mediated pathway seems to be primarily involved in the elimination of cells which have been recognised as foreign, Fas-mediated cytotoxicity seems to play a role in down-regulating immune responses [10,11]. In this instance, Fas–FasL interactions may mediate the removal of the responding CTL population so that huge numbers of CTL do not accumulate over time. Additionally, the tumour necrosis factor receptor, which is related to Fas and also contains a death domain, has been implicated in the control of CTL number following an immune response [12].

2.3. Cytolytic proteins

As mentioned above, the cytolytic proteins reside in the CTL granules, where they await exocytosis towards a target cell. What are these proteins? The first granule function-related protein identified was the pore-forming protein perforin, which was shown to be able to induce calcium-dependent lysis of target cells [13–15]. In the granules, which are acidic, perforin is found in monomeric form in association with proteoglycans. Granule exocytosis releases perforin into the intracellular space where it is exposed to both calcium and neutral pH. The neutral pH causes the release of perforin monomers from proteoglycans [16], allowing the monomers to bind the lipids of the target cell membrane [17,18]. Perforin then inserts into the cell membrane and aggregates in a calcium-dependent manner to form a pore which can be seen by electron microscopy [19,20]. Since perforin is able to cause lysis of a number of cell types, it was originally believed that formation of the perforin pore was sufficient to induce target cell death. However, perforin alone cannot induce target cell DNA fragmentation [21–23], suggesting that perforin cannot mediate all of the events involved in CTL-mediated cytotoxicity. It is now believed that the primary function of perforin is not in cytolysis but rather to allow other cytotoxic mediators to enter the target cell.

The second set of cytolytic proteins identified were the granzymes, a family of CTL-specific serine proteases [24] which co-localise with perforin to the granules [25,26]. Although evidence is only just beginning to accumulate regarding the roles of the granzymes during CTL attack, it has been known for some time that protease inhibitors can protect target cells from cell-mediated lysis [27,28]. Furthermore,

loading of cells with chymotrypsin, trypsin or proteinase K has been found to cause cell lysis, accompanied in most cases by DNA fragmentation and nuclear damage [29]. This suggests that proteases are involved in the induction of target cell death and that granzymes most likely exert their effect inside the target cell, although this latter point awaits confirmation.

An RNA-binding protein called TIA-1 has also been localised to CTL granules [30] and has been shown to induce DNA fragmentation in permeabilised cells [31], suggesting a role for this protein in target cell death. Interestingly, a related but ubiquitously expressed protein called TIAR, has been found to be translocated from the nucleus to the cytoplasm in the target cell during Fas-mediated cytotoxicity [32] a conserved mechanism of inducing cell damage between the Fas and granule killing pathways.

2.4. Target cell death

During both granule- and Fas-mediated cytotoxicity, a target cell dies by undergoing a series of events termed apoptosis. Apoptosis was originally described as a morphological phenomenon occurring in two distinct phases [33,34]. In phase I, the cell's cytoplasm condenses but ribosomal and mitochondrial morphology is maintained. The chromatin condenses and forms crescent-shaped aggregates lining the nuclear membrane, and the nucleolus fragments. During this phase the cell's DNA is fragmented into oligonucleosomal-sized pieces. Eventually, the nucleus breaks up into several fragments, the plasma membrane invaginates and the cell separates into a number of membrane-bound fragments containing morphologically normal mitochondria and other organelles. During phase II these ''apoptotic bodies'' are phagocytosed by neighbouring cells or by macrophages.

Until recently, the biochemical events involved in the induction of apoptosis, and how they are linked to CTL-induced death, were unknown. Much of this recent work has focused on a family of cysteine proteases related to the mammalian interleukin-1β converting enzyme (ICE) and the product of the *Caenorhabditis elegans* ''death gene'' *ced-3*, and has been reviewed elsewhere [35,36]. A number of ICE/Ced-3 proteases have been identified and implicated in cell death. These proteins are generated as inactive precursors requiring proteolytic cleavage for activation. Recently, a link was established between apoptosis and CTL-mediated cytotoxicity by the finding that granzyme B can cleave and activate a number of ICE/Ced-3 proteases [37–46]. Furthermore, this activity is directly related to the ability of granzyme B to induce target cell DNA fragmentation [47,48]. ICE/Ced-3 proteases have also been implicated in Fas-mediated killing [49–56] and, interestingly, an ICE/Ced-3 protease has been found to associate with the ''death domain'' of Fas following receptor oligomerisation [42,57]. It is believed that recruitment of the protease to the oligomerised receptor in some way results in protease activation. This protease is then thought to initiate a cascade of events resulting in target cell death (Fig. 2).

Thus, it seems that the CTL utilises a target cell's endogenous suicide program to induce target cell apoptosis. However, some questions remain. Only granzyme B has been shown to interact with the apoptotic pathway and this mechanism seems to lead

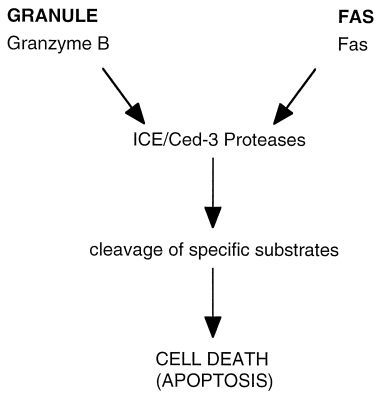

Fig. 2. Target cell death. During granule-mediated cytotoxicity, granzyme B enters the target cell, where it cleaves and activates multiple ICE/Ced-3 proteases. In Fas-mediated killing, an ICE/Ced-3 protease precursor interacts directly with oligomerised receptors, resulting in protease activation. Once activated, ICE/Ced-3 proteases cleave a number of cellular proteins, including poly(ADP-ribose) polymerase DNA-dependent protein kinase, the 70 kDa protein component of the U1 ribonucleoprotein particle, the cytoskeletal proteins actin, fodrin, Gas2, lamins, the GDP dissociation inhibitor D4-GDI, the cell cycle proteins retinoblastoma and the PITSLRE kinases, and the transcription factors sterol regulatory element binding proteins. The end result of ICE/Ced-3 protease activation is target cell death by apoptosis.

predominantly to DNA fragmentation. How membrane damage and cytolysis are induced, and the exact roles of other cytolytic proteins such as granzyme A and perforin, remains to be determined.

2.5. Serial killing

CTL are able to sustain their killing potential over a period of time and are therefore capable of killing a number of target cells. How is this accomplished? Do they secrete all their granules and then rapidly replace them? Do they secrete only a portion of their granules per recognised target cell? Or do CTL switch between granule-mediated and Fas-mediated cytotoxicity in order to maintain their cytotoxic potential?

It now seems clear that CTL maintain cytotoxic potential through the same mechanisms which allow them to be cytotoxic in the first place. As outlined above, the initial recognition of a target cell through the TcR triggers the synthesis of cytolytic proteins, which are stored in the granules. Upon further interaction with a target cell, the CTL is triggered to degranulate. However, concurrent with degranulation, the CTL also synthesises new lytic proteins which presumably replenish the granules [58]. Surprisingly, approximately 30% of these newly-synthesised proteins are not stored in the granules, but rather are constitutively secreted from the cell, which may account for some bystander lysis (that is, killing of cells not specifically recognised by CTL). Since new synthesis of lytic proteins is initiated in conjunction with degranulation, this provides an excellent mechanism by which serial killing may be accomplished. Additionally, FasL expression is also triggered by TcR engagement, allowing this mechanism of killing to be maintained across multiple targets.

Therefore, it seems that both granule- and Fas-mediated killing are maintained throughout serial killing as a direct result of TcR engagement. Thus, it is unlikely that a CTL ''switches'' between killing mechanisms in order to maintain cytolytic potential. Rather, it is likely that each cytotoxic mechanism serves a different purpose. That is, granule-mediated killing is responsible for destroying specifically-recognised foreign cells while Fas-mediated killing is responsible for destroying self cells at the end of an immune response.

3. The lytic granule as a lysosome

3.1. Lysosomal contents

The cytolytic proteins are not the only constituents of lytic granules. Also present are proteins more classically defined as lysosomal components, for example, Cathepsins B and D, α-glucosidase, LAMP1 and LAMP2 [59]. Lysosomes have a heterogeneous morphology between cells and even within the same cell type. Classically they have been defined as dense organelles that contain acidic hydrolases which degrade intracellular components and macromolecules that have been endocytosed [60]. Both immunofluoresence and immuno-electron microscopic studies indicate co-localisation of lysosomal and lytic proteins in CTL, indicating that the lytic granules also function as lysosomes [61,62].

3.2. Lytic granules are acidic compartments

Like lysosomes, the lytic granules of CTL are acidic organelles [61–63]. Evidence for the presence of a proton pump was provided when an inhibitor of vacuolar H^+-ATPase (concanamycin A) resulted in a raised pH and altered granule morphology [64]. This treatment also inhibits the cytolytic activity of the CTL, demonstrating the importance of granule acidification for CTL function.

Interestingly, the low pH of the granule only provides a favourable environment for the activity of the lysosomal hydrolases localised there (reviewed [65]). The cytolytic proteins, perforin and granzymes, are optimally active at a higher pH than that found in the granule. Only upon granule exocytosis and the release of contents to the extracellular environment are these proteins exposed to a more neutral pH at which they are active. The storage of these proteins in an acidic environment in which they are not active may be one mechanism utilised by the cell to protect itself against their lytic activity. Additionally, as described in Section 2.3, the proteoglycan, Chondroitin sulphate A, which is a major component of granules, interacts with both granzymes and perforin within the granule [66,67]. There is evidence that Chondroitin sulphate A binds to and inhibits perforin in a pH dependent manner [67]. Thus, this interaction is

thought to maintain lytic proteins in an inactive state while stored in the lytic granules.

Hence, the function of lytic granule content is determined by pH: the granule itself maintains an acidic pH thus allowing for the activity of lysosomal hydrolases, while release of the lytic granule contents into the neutral pH of the extracellular space by exocytosis activates the cytolytic proteins.

3.3. The lytic granule as an endocytic compartment

One criterion of lysosomal function would be the ability of lytic granules to receive and degrade endocytosed macromolecules from the extracellular environment (see Fig. 3). Delivery has indeed been observed. Endocytosed cationized ferritin (CF) accumulates in lytic granules with kinetics similar to that of delivery to lysosomes [61]. This uptake was also sensitive to low temperatures which suggests that the granule is a late compartment on the endocytic route.

The mature lytic granule does not contain the mannose-6-phosphate receptor (M6PR), a protein associated with endosomes but not with lysosomes (see Fig. 3 and Section 4.1). Although granule proteins, synthesised in newly activated CTL, are initially found in a compartment that is M6PR positive, at a later stage in the biogenesis of the organelle they are found to be present in a M6PR negative compartment. This observation also implies that the lytic proteins are present in a compartment late in the endocytic pathway.

Thus, the data suggest that the lytic granule can perform two functions for the CTL that are, in other cell types, usually carried out by two distinct or-

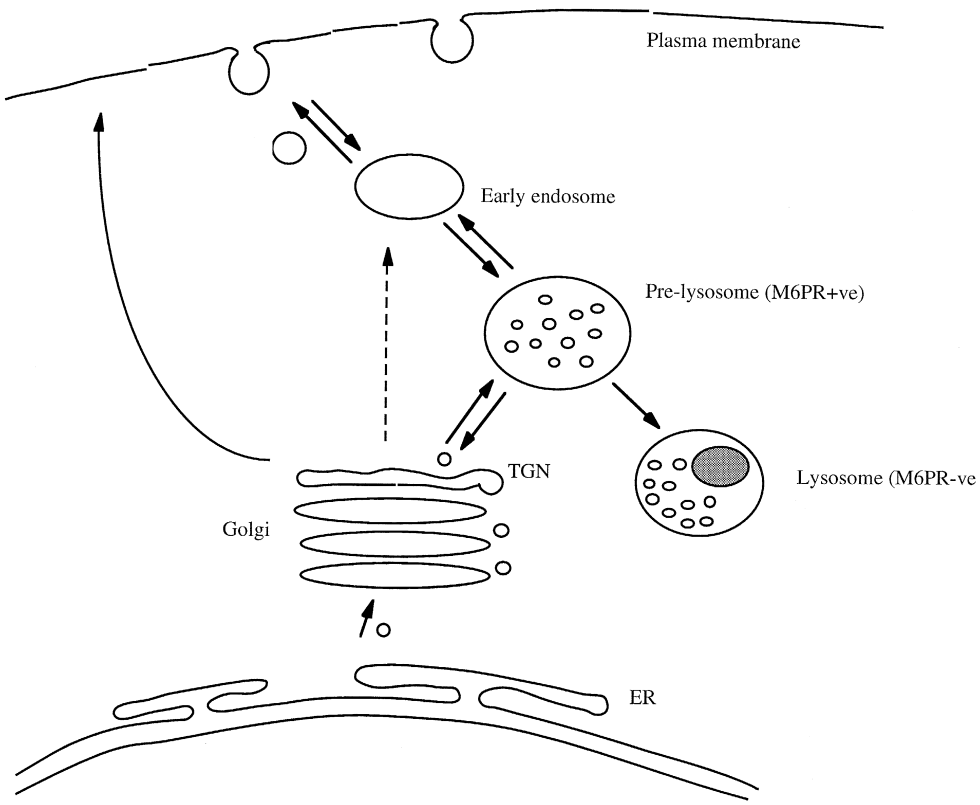

Fig. 3. Biosynthetic and endocytic pathways. Newly synthesised proteins are transported from the endoplasmic reticulum (ER) through the Golgi to the *trans* Golgi network (TGN). During this transportation, soluble lysosomal proteins aquire a mannose-6-phosphate tag which enables association with the mannose-6-phosphate receptor (M6PR). At the TGN, both receptor and ligand are selectively included into clathrin coated vesicles and are transported to a pre-lysosomal compartment. The ligand dissociates from the receptor in this compartment, allowing the M6PR to recycle back to the TGN. Extracellular macromolecules, which bind to receptors at the plasma membrane that are specifically included into clathrin coated vesicles, are endocytosed and transported to an early endosome. From there, proteins can either recycle back to the plasma membrane or continue on along a pathway to the lysosome.

ganelles. The lysosomes of CTLs are therefore not only involved in internal degradation events but, uniquely, can be exocytosed as a regulated event to allow secretion of contents whose function is to destroy infected/cancerous cells.

4. Lytic granules as secretory organelles

In addition to the mechanisms that are needed to accomplish this ''double life'' of the lytic granules outlined earlier, a CTL must be able to target both newly synthesised lysosomal and cytolytic proteins to the same compartment. This is very different from the situation in ''conventional'' secretory cells, where lysosomal and secretory proteins are sorted to different organelles which maintain separate functions.

4.1. Sorting of lysosomal proteins

For soluble proteins, two distinct pathways for sorting to the lytic granules have been described. The major pathway responsible for targeting of soluble lysosomal hydrolases to the lysosome utilises the mannose-6-phosphate-receptor (M6PR) [68]. Proteins destined for the lysosome are covalently modified by the addition of mannose-6-phosphate (M6P) groups to their N-linked glycans in the *cis*-Golgi network. The phosphotransferase that catalyses this reaction recognises a specific signal in the polypeptide chain of each lysosomal hydrolases. Although this signal has been defined for some hydrolases [69], there is no simple motif that is shared between the different hydrolases. Proteins that have acquired the M6P tag are able to interact with the transmembrane M6PR in the TGN [70]. Both receptor and ligand enter into clathrin coated vesicles and are thus, selectively transported to a prelysosome compartment. Exposure to the low pH of the prelysosome results in the dissociation of lysosomal hydrolases from the M6PR, which can then recycle back to the TGN.

However, delivery of lysosomal proteins is not solely dependent on M6P-tagging and M6PR. A M6PR-independent route also exists. Studies on cells derived from patients with I-cell disease (mucolipidosis II) have shown that lysosomal hydrolases can still reach the lysosome in these cells despite the fact that none of the hydrolases acquire the M6P modification. In I-cell disease, the phosphotransferase responsible for the phosphorylation of mannose residues is absent or defective. Consequently, lysosomal proteins do not acquire the M6P group and are not, therefore, transported to the lysosome by the M6PR. Instead, they appear to be secreted from the cells via the constitutive pathway. Thus, the observation that a fraction of lysosomal enzymes are present in lysosomes suggests that, in addition to the well characterised M6PR pathway, an alternative, M6PR-independent route to the lysosome also exists [69].

In contrast to the soluble lysosomal proteins, the transport of integral membrane proteins to lysosomes does not involve the M6PR. Instead, the cytoplasmic tail of these proteins is necessary for their delivery to the lysosome. Two types of signal have been defined which are able to mediate targeting to the lysosomes, a tyrosine based signal and a di-leucine based motif (reviewed by W. Hunziker and H. Geuze, [71]). These two lysosomal sorting signals have also been identified in the lytic granule proteins of CTL, such as T1A, CD3γ and δ chains of the TCR and CD63. The tyrosine based motif has been shown to interact with the clathrin-associated protein complexes AP1 and AP2, indicating that clathrin coated vesicles are used for the transportation of these lytic granule proteins [72].

4.2. Sorting of granzymes and perforin

How are the soluble secretory proteins, granzymes and perforin, sorted to the lytic granules? In conventional secretory cells, the secretory proteins are sorted by a selective aggregation in the TGN, which results in the segregation of these proteins to a distinct organelle [73].

The sorting mechanism of granzymes became clear from studies on CTL derived from patients with I-cell disease. In these cells, granzymes A and B were not correctly sorted, but were secreted constitutively. This suggested that granzymes A and B were normally targeted to the granules by the M6PR pathway, and that in I-cells, where the M6P is not added, the granzymes cannot be sorted correctly. Biochemical data confirmed this, demonstrating that granzymes do in fact bear M6P residues in wild type cells [74]. Nevertheless, approximately 20% of the granzymes in I-cells are still properly localised to the lytic

granules, indicating that granzymes can also use an M6PR-independent pathway. Whether this M6PR-independent pathway is the same as that used by the lysosomal hydrolases remains to be determined.

Although perforin is also a soluble secreted protein, it is not sorted to the lytic granules by the mannose-6-phosphate receptor. Neither rat [75] nor human [76] perforin acquire a M6P modification during their biosynthesis, excluding the possibility of sorting via this route. The observation that I-cell CTL are able to lyse target cells with the same efficiency as wild-type CTL supports the idea that perforin is targeted normally in these cells, and that only MPR-independent mechanisms are responsible for correct packaging and secretion of perforin [74].

5. Secretory lysosomes in the haemopoietic lineage

5.1. Other cell types with secretory lysosomes

The lytic granules of CTL and NK cells exhibit all the characteristics of conventional lysosomes. In addition, lytic granules are able to fuse with the plasma membrane and undergo regulated exocytosis acting as secretory organelles (reviewed in [77]). For a CTL, this process of secretion is of crucial importance to it's function because this is how the cytotoxic proteins perforin and granzymes, which are stored in the lytic granules, get to their site of action.

Secretory lysosomes are not unique to cytotoxic T lymphocytes but are found in other cells types, for example eosinophils, neutrophils, basophils, platelets, mast cells and macrophages (reviewed [77]). Interestingly, these cells all belong to the same lineage, the haemopoietic lineage. This introduces the possibility that these cells express lineage specific proteins that are responsible for the regulated exocytosis of their lysosome. Thus, it might be expected to find lineage specific expression of proteins that have a role in, for example, membrane recognition and fusion, such as v- and t-SNARES, rabs or perhaps a protein that acts as a calcium sensor to regulate secretion such as the role proposed for synaptotagmin in neurons. Recent work has demonstrated that lysosomes in fibroblasts can also undergo exocytosis in response to a calcium flux generated by ionomycin [78]. This work suggests that lysosome fusion with the plasma membrane may be possible in all cell types (perhaps providing a mechanism for membrane repair). The haemopoietic lineage may have exploited this ability to undergo membrane fusion to generate a regulated secretory organelle.

5.2. Chediak Higashi Syndrome

The recessive, human genetic defect that results in Chediak Higashi Syndrome (CHS) in homozygous patients may provide information on how these specialised cells allow lysosome secretion. Although this defect affects most cells, resulting in enlarged lysosomes, in non-haemopoietic cells there does not appear to be any functional impairment. However, in haemopoietic cells secretion of lysosomes no longer occurs [79]. There are several possibilities for the nature of this genetic defect since multiple steps are required for secretion to proceed. Firstly, the T cell receptor (TcR) signalling pathway has to be activated in order to produce the increase in internal Ca^{2+} concentration required for exocytosis of the secretory lysosome. TcR signalling is functional, at least to some extent, since resting CHS cells can be triggered via the TcR to proliferate and develop into mature cells. The next event to occur is movement of the granule towards the plasma membrane, so that it is close to the point of contact with the target cell. This process is dependent on microtubules. Work by Perou and Kaplan [80] indicates however, that there is no microtubule associated defect in CHS fibroblasts. Polarisation of CHS granules against the plasma membrane at the site of contact with the target cell has been observed at IF level (unpublished observation), however, a more detailed study with electron microscopy is required. It is possible that the large size of the mutant secretory granule simply hinders its movement through the cytoplasm thus preventing its contact and therefore fusion with the plasma membrane. Certainly, experiments where macrophages were fed beads of various sizes to increase the size of the lysosome indicated that the larger the bead internalised, the more static the organelle was [80]. However, an argument against this comes from the observation that CHS mast cells can be stimulated to secrete by using a Ca^{2+} ionophore [81].

The last step of secretion is fusion of the granule with the plasma membrane. Since an abnormally

large organelle is formed in the mutant cells, it has been proposed that a membrane fusion event may be affected. Interestingly, during the biogenesis of the secretory organelle in newly stimulated CHS cells, there are initially ~50 M6PR positive granules per cell and these cells look identical to wild type cells. However, as the CHS cell matures, the number of granules decreases to about 1–3 M6PR negative granules per cell, in contrast to wild type cells where the initial number is maintained (L.J.P. and G.M.G., unpublished observation). This suggests that as mature lysosomes are formed they fuse to produce fewer, but larger organelles.

The gene responsible for the defect has now been identified [82,83]. The gene encodes several alternatively spliced products encoding proteins predicted to be in the range of 200 kDa. The gene shows no regions of strong homology to other sequences, and gives no clue as to it's role in secretion.

6. Summary and conclusion

CTL are important cells in the immune system which are able to recognise and directly destroy virally infected, tumorigenic or foreign cells. The proteins which mediate this destruction are packaged into specialised secretory granules, termed lytic granules, which are secreted in response to target cell recognition. Curiously these specialised secretory granules also contain all the lysosomal hydrolases, and in CTL the lytic granules serve two separate functions: as a lysosome within the cell, and as a secretory granule when a target cell is recognised.

These ''secretory lysosomes'', which serve important roles in both protein degradation within the cells as well as regulated secretion of proteins from the cells, are also found in other cell types, all of which are derived from the hemopoietic lineage. This observation raises the possibility that cells of the hemopoietic lineage possess specialised sorting and secretory mechanisms which allow the lysosomes to be used as secretory organelles. Studies on Chediak Higashi syndrome support this idea, since in this naturally occurring genetic mutation, cells with secretory lysosomes are unable to secrete their granules while other conventional secretory cells are able to do so. Further studies on the mechanisms which regulate secretion of lytic proteins from CTL should identify the proteins involved in this unusual secretory pathway.

Some aspects of the differences between conventional and ''secretory'' lysosomes remain unresolved. How the biogenesis of the secretory lysosome differs from that of a conventional secretory granule is unclear. While conventional secretory cells sort proteins destined for the granule by a selective condensation in the TGN, the secretory lysosomes seem to use a combination of lysosomal and other sorting signals. Our preliminary studies suggest that haemopoietic cells possess specialised sorting mechanisms which allow the correct sorting of the secreted products to the lysosome, and that these signals are different from those found in conventional secretory (e.g. neurosecretory) cells. This finding and the observation that fibroblast lysosomes can undergo calcium-mediated exocytosis suggests that the unusual secretory system found in haemopoietic cells may be a result of specialised sorting mechanisms in these cells. In this case the Chediak lesion may turn out to be a sorting defect.

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