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Immunology

Delineation of the subunit composition of human proteasomes using antisera against the major histocompatibility complex-encoded LMP2 and LMP7 subunits

(related subcomplexes/two-dimensional gel analysis/precursors)

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ABSTRACT **The products of the *Lmp2* and *Lmp7* genes located in the major histocompatibility complex (MHC) class II region are postulated to form part of the proteasome complex. This large, multisubunit complex forms the major, nonlysosomal proteolytic machinery for the degradation of endogenous proteins and has been suggested to play a role in the processing of antigens presented by MHC class I molecules. The role of the MHC-encoded subunits within the proteasome has however remained enigmatic. To study this role, we have raised antibodies to recombinant LMP2 and LMP7 proteins. Under denaturing conditions, the anti-LMP7 serum recognizes one subunit of proteasome, whereas the anti-LMP2 serum recognizes two subunits, which may represent different forms of LMP2. The specificity of these sera has been ascertained by a lack of reactivity in T2 cells, which lack both genes. Furthermore under native conditions the anti-LMP2 serum immunoprecipitates a complex that is similar to proteasome but lacks several subunits, including LMP7. Preclearing experiments using this serum and a monoclonal antibody (2-17) specific for the non-MHC-encoded C2 proteasome subunit demonstrate that the complexes recognized by these two sera are distinct and that four subunits are unique to the complex precipitated by the anti-LMP2 serum. Interestingly, the different forms of LMP2 are segregated between these complexes. The relationship of the two complexes is discussed.**

The immune system utilizes two postulated pathways to present foreign antigens to T cells (1). Major histocompatibility complex (MHC) class II molecules, after synthesis and assembly within the endoplasmic reticulum, are transported to a lysosomal or endosomal compartment, where they bind peptides generated from endocytosed or phagocytosed exogenous antigens. The binary complex subsequently formed is translocated to the cell surface, where it is recognized by the appropriate helper T-cell receptor. MHC class I molecules display peptide fragments derived from endogenous proteins, such as viral antigens, to cytotoxic T lymphocytes. These fragments are proposed to originate in the cytosol from which they are transported into the endoplasmic reticulum lumen, where they bind class I molecules. Several studies have implicated genes within the MHC class II region to play a role in this pathway. Two genes, *Tap1* and *Tap2*, encode transmembrane proteins (TAP, transporters associated with antigen processing) that are part of the ATP-binding-cassette-containing family of transport proteins (2–5) and are postulated to transport endogenous antigenic peptides into the endoplasmic reticulum lumen (6–9). Two genes, in close proximity to *Tap1* and *Tap2*, designated *Lmp2* and *Lmp7* encode proteins (LMP, low molecular mass polypeptide) highly homologous to subunits of proteasome (10–12). Proteasomes are ubiquitous, multisubunit proteases composed of 15–20 distinct, noncovalently associated subunits of low molecular weight (13). Proteasomes exhibit at least three separate endoprotease activities and are proposed to form the major route for nonlysosomal intracellular protein turnover in eukaryotic cells. They are proposed to assemble into a supercomplex upon association with other subcellular factors in an ATP-dependent process (14). Thus the proteasome is often referred to as the 20S proteasome and its larger relative as the 26S ubiquitin-conjugate-degrading enzyme complex whose function is believed to be the ATP-dependent degradation of proteins conjugated to ubiquitin (15).

In the mouse, the LMP2 and LMP7 proteins have been shown to be part of the LMP complex, which is a serologically and structurally distinct family of "proteasomes" (16). The mapping of the two genes to the MHC, their interferon γ (IFN-γ) induction (17), and their similarity to proteasomes (18) make both LMP, proteasome, and any other related complexes attractive candidates as the processors of endogenous antigens.

In this paper we utilize antibodies against LMP2 and LMP7 to investigate the structure of human proteasome complexes and to delineate the presence and function of the MHC-encoded subunits in these complexes. The results demonstrate the existence of two forms of LMP2, which are segregated into two proteasome-like complexes, one of which lacks LMP7. The possible relationship of these complexes is investigated.

MATERIALS AND METHODS

Cloning, Expression, and Purification of LMP2 and LMP7 Recombinant Proteins. Full-length human cDNAs corresponding to LMP2 and LMP7 were isolated from a human B-lymphoblastoid cell line (WT51) λgt10 library by standard methods (19). For LMP2, a fragment starting at the initiator ATG was subsequently subcloned into the pRSET vector (Invitrogen), which tags a fusion fragment containing a six-histidine stretch onto the N terminus of the expressed protein, and this was used to generate and purify LMP2 fusion protein. Briefly, clones expressing the appropriate protein were grown, harvested, lysed in 6 M guanidine hydrochloride (pH 8.0), and fusion proteins were isolated from a cleared lysate using Ni^{2+}-chelate affinity chromatography. For LMP7, an *Eco*RI fragment from bases 362–1307 (ref. 10; containing the complete open reading frame) was used for subcloning into the pRSET vector. In both cases, single

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Abbreviations: 2D, two-dimensional; mAb, monoclonal antibody; IFN-γ, interferon γ; MHC, major histocompatibility complex.

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protein bands corresponding to the correct molecular weight were observed.

Western Blot Analysis. For Western blot analysis, cells (2–5 × 10^4 per well) were resuspended in SDS sample buffer (0.125 M Tris·HCl, pH 6.8/1% SDS/1% 2-mercapoethanol) prior to SDS/PAGE. Proteins were transferred onto nitrocellulose by standard methods and probed with 1:1000 dilutions of anti-LMP2 or anti-LMP7 serum or monoclonal antibody (mAb) 2-17 (1 μg/ml). Bound antibodies were detected using peroxidase-conjugated secondary antibodies and diaminobenzidine in the presence of NiCl (20).

Metabolic Radiolabeling and Immunoprecipitation. For immunoprecipitation, 10^7 cells were metabolically labeled in 2 ml of RPMI (minus methionine) with 300–500 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (Amersham) for 5 h. After washing in phosphate-buffered saline the cells were lysed in 0.5 ml of lysis buffer [1% Nonidet P-40/50 mM Tris·HCl, pH 7.4/150 mM NaCl/1 mM phenylmethylsulfonyl fluoride/aprotinin (30 μg/ml)]. Lysates were preabsorbed with 25 μl of normal rabbit serum and 100 μl of protein A-Sepharose beads (Pharmacia; 1:1 slurry in lysis buffer) for 1 h. Specific precipitation was done for 1 h with 10 μg of mAb or 10 μl of polyclonal serum. Precipitates were collected by incubation for 30 min with 100 μl of protein A-Sepharose slurry and centrifugation. For precipitations with the mAb, 10 μl of a rabbit anti-mouse immunoglobulin preparation (Dako) was added to the lysate for 30 min prior to precipitate collection. After washing three times in lysis buffer containing 0.25% Nonidet P-40, the precipitates were analyzed by nonequilibrium pH gradient electrophoresis (using pH 3.5–10 Amholines; Pharmacia) in the first dimension and SDS/PAGE (11%) in the second dimension.

Preclearing Experiments. For preclearing of cell lysates, 50 μl of anti-LMP-2 antiserum was incubated for 1 h with 0.5 ml of lysate, and the precipitate was collected 30 min after adding 100 μl of protein A-Sepharose slurry. An additional 50 μl of serum was added, and then cycles of protein A-Sepharose slurry additions were repeated until the measurable cpm dropped to negligible levels. The samples were divided in half and subsequently used for immunoprecipitations with the anti-LMP2 antiserum and mAb 2-17, respectively, as described above. For the converse experiment, 10 μg of mAb was utilized to preclear cell extracts as above. The sample was divided and used for immunoprecipitations with mAb 2-17 and anti-LMP2 antiserum.

Immunoprecipitation of LMP2 and LMP7 from Denatured Proteasome Extracts. Immunoprecipitation was performed with the mAb 2-17 as described above. The pellet was resuspended in lysis buffer containing 1% SDS and left overnight. The sample was diluted with lysis buffer to a final SDS concentration of 0.05% and incubated for 1 h at 4°C prior to immunoprecipitation. Precipitates were washed three times and resuspended in SDS sample buffer for SDS/PAGE or washed 10 times before suspension in nonequilibrium pH gradient electrophoresis buffer.

RESULTS

Characterization of Antisera. Assessment of anti-LMP2 and anti-LMP7 sera specificity by Western analysis (Fig. 1) revealed that each serum recognizes a single protein of the expected size in MWF (a normal homozygous B-lymphoblastoid cell line) cell lysates (lanes 1 and 4). In addition, these bands are IFN-γ inducible in HeLa and other cell line lysates (lanes 11–16), a known property of *Lmp2* and *Lmp7*, and are absent in T2 cell lysates (lanes 2 and 5). The T2 mutant cell line contains a homozygous deletion spanning almost the entire MHC class II region and therefore lacks *Tap1*, *Tap2*, *Lmp2*, and *Lmp7*. The T1 cell line is derived from the same parental line as T2 but contains a functional class II region

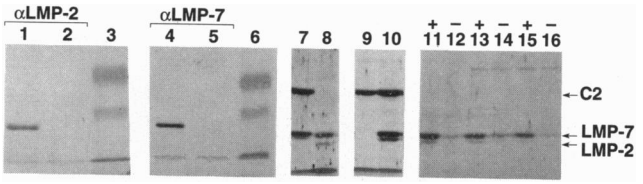

FIG. 1. Western analysis using anti-LMP2 (αLMP2), anti-LMP7 (αLMP7), and mAb 2-17. Cell lysates were separated by SDS/PAGE and probed with sera and developed as described. Lanes 3 and 6, markers [18 kDa (running with the dye front in this case), 28 kDa, and 30 kDa]; lanes 1, 4, 7, and 10, MWF cell lysate. Lanes 2, 5, and 9, T2 cell lysate; lane 8, WEHI mouse cells. Lanes 11 and 12, HeLa cell lysate; lanes 13 and 14, PANC-1 cell lysate; lanes 15 and 16, Hepa-G2 cell lysate. Lanes 1 and 2 were probed with anti-LMP2, and lanes 4 and 5 were probed with anti-LMP7 as indicated. Lanes 7–10 were probed with a mixture of all three antibodies. Lanes 11–16 were probed with anti-LMP2 and anti-LMP7 only; the absence (−) or presence (+) of IFN-γ induction is indicated above the lane numbers.

(21). These results suggest a high degree of specificity of the sera, which is important in view of the known homology of LMP2 and LMP7 to other subunits of the proteasome (22). The mAb 2-17 recognizes a single protein of the expected molecular weight, which is also found in T2 cells, indicating that it is not encoded by a gene in the MHC class II region.

Identification of LMP2 and LMP7 Proteins in Two-Dimensional (2D) Gel Profiles of Proteasome. Comparison of the 2D gel profiles of the proteasomes immunoprecipitated by the mAb 2-17 from T1 and T2 cells reveals that two subunits are missing in the mutant line (Fig. 2). In addition, a subunit missing in T1 but found in MWF (Fig. 3*A*) and other normal B-lymphoblastoid lines (data not shown) is also missing in T2 cells. In addition, subunit X is unique to the T1 and T2 lines, and subunit Y is found at a greater intensity in these lines as compared to other normal B-lymphoblastoid lines. The former finding is unique to our profiles, whereas the latter agrees with previous reports (23). These differences may be explained by the fact that T1 and T2 are derived from T × B cell fusions, although it is intriguing that the subunit missing in T1 corresponds to LMP-2b (see below). The presence of subunit X in only our immunoprecipitates may reflect the loss of this subunit due to antibody-induced perturbations when other antibodies are used. To delineate which subunits correspond to the LMP2 and LMP7 proteins, proteasomes precipitated by the mAb 2-17 were denatured in SDS, and the anti-LMP2 and anti-LMP7 sera were used to immunoprecipitate from these extracts after the SDS concentration had been reduced by dilution. A single protein was precipitated by the anti-LMP7 serum, whereas the anti-LMP2 serum identifies two proteins (Fig. 4). The protein recognized by the LMP7 antibodies is indicated in Fig. 3 and agrees with previous reports (24). The two proteins isolated by the anti-LMP2 serum correspond to the spots marked in Fig. 3 as LMP2a and LMP2b, as determined by 2D gel analysis (Fig. 4). The lower of these spots (LMP2b) has been suggested to correspond to LMP2 based on transfection of HeLa cells with mouse *Lmp2* cDNA (24). In our hands, the mouse and human LMP2 migrate differentially upon electrophoresis despite their predicted similar mobility based on protein sequence (Fig. 1, lane 8). However, we suggest that both spots represent different forms of LMP2 on the following basis: First, both are absent in T2 cells. Second, both are recognized by the anti-LMP2 serum under denaturing conditions. Finally, the mouse homologues of LMP2a and LMP2b both show an identical migration shift (a reflection of changes in charge produced by polymorphic amino acid residues) on 2D gel analysis of LMP complex from cells of different haplotype, which are known to be polymorphic for the *Lmp2* locus (25). LMP2 has been reported to be processed probably by the

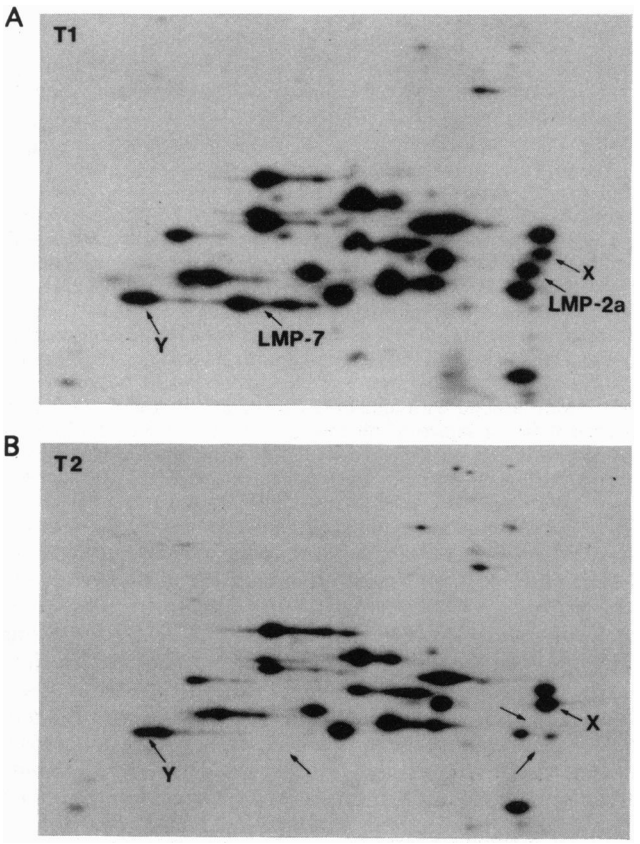

FIG. 2. 2D gel analysis of proteasomes from T1 (*A*) and T2 (*B*) cells. Immunoprecipitates were obtained as described, and proteins were separated by 2D PAGE. Dried 2D gels were exposed to X-OMAT film for 1 or 2 days. Subunit X is unique to T1 and T2, as compared to other lymphoblastoid lines, and Y is found at a greater intensity. Arrows in *B* indicate subunits missing in T2 but present in T1 and other lymphoblastoid lines. 2D profiles are orientated basic pI (left) to acidic pI (right).

posttranslational removal of the first 20 amino acids (24), and *in vitro* translation of mouse *Lmp2*, using a system that allows no processing, results in a product that on 2D gel analysis comigrates with the mouse equivalent of the upper subunit (25). The upper and lower proteins migrate very closely to the correct size and charge predicted for the LMP2 unprocessed and processed forms (23.3 kDa, pI 4.89; 21.3 kDa, pI 4.65). Thus, we conclude that LMP2a represents the unprocessed form of LMP2, and LMP2b represents the same protein after the removal of the N-terminal 20 amino acid residues.

A Subpopulation of Proteasomes Is Precipitated by Anti-LMP-2 Serum. A typical 2D gel profile of proteasomes precipitated from the homozygous B-lymphoblastoid cell line MWF with mAb 2-17 is shown in Fig. 3*A*. Since a collection of proteins ranging in size from 30 kDa to 110 kDa (normally associated with the 26S form) are not precipitated, we assume this represents the 20S form of proteasome. The LMP7 protein often appears as a doublet of spots as do some of the other subunits. Both are assigned as LMP7, since they both are absent in T2 and either represent differentially modified forms of LMP7 or are simply an artifact of the gel system. It may be relevant that two alternatively spliced forms of *Lmp7* mRNA have been identified (24). In our hands, the relative intensities of several subunits fluctuated in different precipitations. This is also seen in the profiles reported in ref. 23. These differences could be a consequence of some autodegradation of the complex or may be due to some of these subunits being more prone to loss from the proteasome due to antibody-induced perturbations. However, these properties make it difficult to ascertain the effects of IFN-γ induction on individual subunits of proteasome.

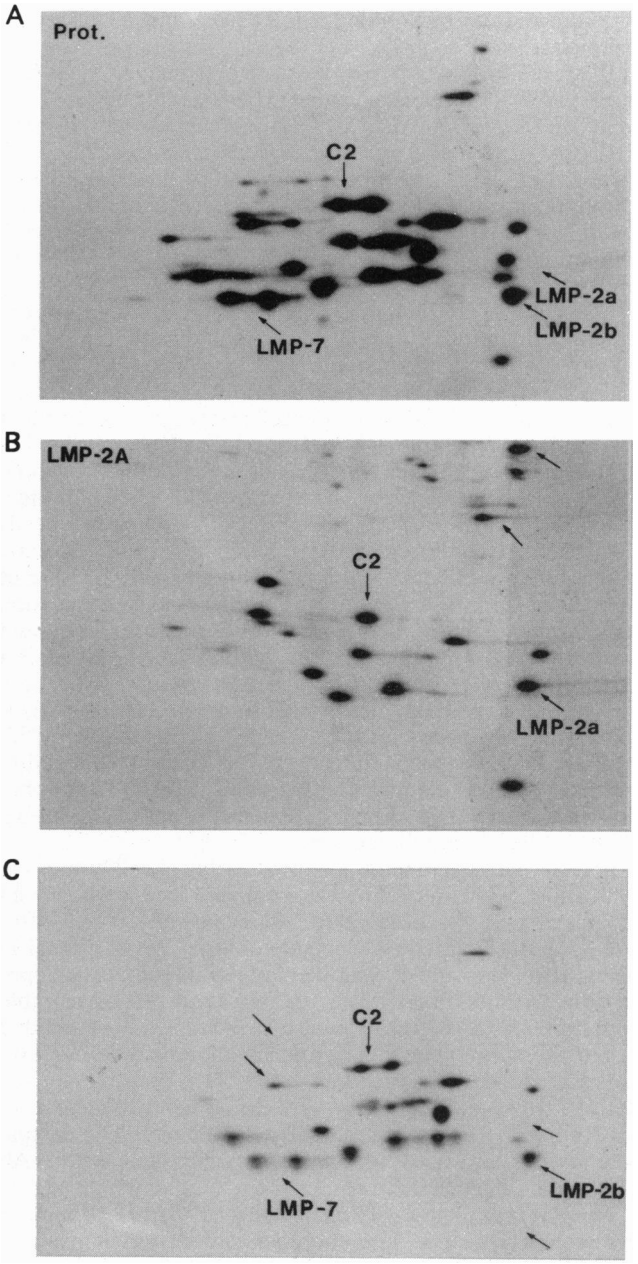

FIG. 3. 2D gel analysis of anti-LMP2 and mAb 2-17 immunoprecipitates. Analysis was performed, and profiles are oriented, as described in Fig. 2. (*A*) mAb 2-17 immunoprecipitate from MWF cells. (*B*) Anti-LMP2 immunoprecipitate from MWF cells. (*C*) mAb 2-17 immunoprecipitate from a MWF cell lysate precleared exhaustively with anti-LMP2. Subunits LMP-2a, LMP2b, LMP7, and C2 are indicated. Unassigned arrows in *B* indicate unknown proteins regularly coprecipitated with LMP2A, and unassigned arrows in *C* indicate subunits that are cleared by anti-LMP2 serum.

Precipitations carried out using the anti-LMP7 failed to precipitate an intact complex or an isolated protein under native conditions. In contrast, the anti-LMP2 serum immunoprecipitated an intact complex under native conditions, which, however, lacks LMP7 and several other subunits as marked in Fig. 3*B*. Interestingly this complex also lacks LMP2b. The original mouse serum used to identify the LMP complex was an alloantiserum (H-2^b anti-H-2^d) made in congenic mice, which apparently recognizes only the LMP2 subunit (11, 17). However this serum precipitates a complex

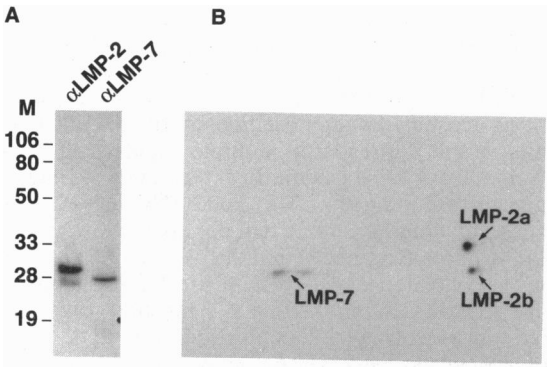

FIG. 4. Precipitation of LMP2 and LMP7 under denaturing conditions. Precipitations were performed as outlined in *Materials and Methods*. (*A*) SDS/PAGE analysis of anti-LMP2 (αLMP-2) and anti-LMP7 (αLMP-7) immunoprecipitates. A protein marker track is shown. (*B*) Anti-LMP2 and anti-LMP7 immunoprecipitates obtained at the same time as those in *A* were mixed and analyzed by 2D gel analysis. 2D profiles are oriented as in Fig. 2. Both samples were run individually to determine corresponding spots (data not shown). The spot assignments are based on this.

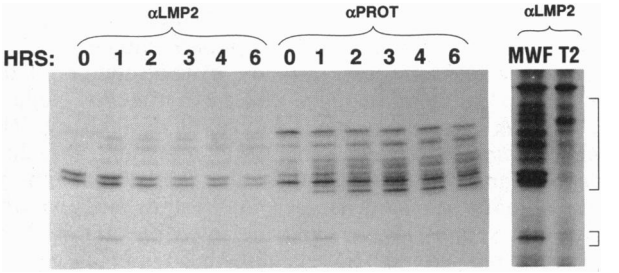

FIG. 5. Pulse–chase analysis using the anti-LMP2 (αLMP2) antisera and mAb 2-17 (αPROT). MWF cells were labeled for 30 min in [^{35}S]methionine-containing medium, washed, and subsequently chased with normal medium for up to 6 h. Aliquots were removed at the times shown following the pulse period, and lysates were prepared. The immunoprecipitates from these lysates were analyzed by SDS/PAGE followed by fluorography. The bands corresponding to proteasome subunits, as determined by comparison of MWF and T2 cells, are bracketed.

containing both LMP2 and LMP7. Therefore we sought to ascertain the significance of the complex precipitated by our anti-LMP2 serum (henceforth referred to as the LMP2A complex).

In all preclearing experiments the protocol outlined in the *Materials and Methods* section was used, and samples from the last preclearing step were analyzed by 2D PAGE to ensure that preclearing was complete. MWF cell lysates precleared exhaustively with the mAb 2-17 and used for immunoprecipitation with the anti-LMP2 serum resulted in no precipitated material (data not shown). Since the mAb 2-17 recognizes human proteasome subunit C2, which is present in both species of complex, this result is expected. Extracts precleared exhaustively with the anti-LMP2 serum were used to perform the reciprocal experiment by immunoprecipitation of these by the mAb 2-17. An intact complex is precipitated, implying that the complex precipitated by the anti-LMP2 serum represents a distinct subpopulation of proteasome. The resulting 2D gel profile is shown in Fig. 3*C*. Interestingly, four subunits, including LMP2a, are removed by the anti-LMP2 serum, suggesting that they are unique to the complex precipitated by this serum. This also suggests that the LMP2A complex is not simply a (antibody-induced) breakdown product of the 20S complex. That these subunits are not lost simply due to degradation was implied by analysis of mAb 2-17 precipitates from extracts that were only partially precleared by one round of preclearing with the anti-LMP2 serum; these same subunits were present but at a noticeably decreased intensity, suggesting that the loss of these proteins is antibody dependent. The precipitation of these subunits in high amounts under normal conditions also excludes the possibility that a proteolytic activity in the anti-LMP2 serum is degrading these subunits in a concentration-dependent manner. These results also suggest that the 2D gel profile seen from mAb 2-17 immunoprecipitations (Fig. 3) is a composite of the two populations.

The same LMP2A complex is identified by the anti-LMP2 serum in several other human cell lines (data not shown). We have detected this complex in a pancreatic epitheloid carcinoma line (PANC-1), in a hepatoma line (HepaG2), in an astrocytoma line, in Jurkat (human T-cell lymphoma) cells, and in a retinoblastoma-derived line (WERI-Rb-1). Interestingly, precipitation in these lines is enhanced by IFN-γ induction, suggesting that the serum is precipitating through a IFN-γ-inducible subunit. In addition, this serum does not precipitate a complex from T2 cells; since the only subunit of the LMP2A complex absent in T2 is LMP2a, the serum must be recognizing a native epitope on LMP2 that is within the presequence or one that is inaccessible when LMP2b is incorporated into the 20S form. To determine whether the LMP2A complex is an assembly precursor of the "mature 20S complex," we performed pulse–chase experiments. Cells were pulsed with [^{35}S]methionine for 30 min, washed, and chased with normal medium for up to 6 h. Lysates were collected at individual time points, and anti-LMP2 immunoprecipitates and proteasome precipitates, obtained under native conditions, were analyzed by SDS/PAGE. Fig. 5 shows the results of such an analysis. Lanes 1–6 indicate that the anti-LMP2 serum precipitates a complex after the pulse period. The subunit intensities of this LMP2A pattern decrease slightly, although the complex is still present after a 6 h chase. The identity of the bands in this profile is unknown, and the individual bands may correspond to several subunits. Similarly the 2-17 mAb precipitates a complex after the pulse period. The intensity of several bands increases during the chase period, reflecting assembly, although the pattern does not change significantly after 2 h (lanes marked αProt 0–6). The final two lanes are anti-LMP2 immunoprecipitates from MWF and T2 cells and demonstrate that the bracketed bands correspond to proteasome subunits by their absence in the T2 cell lysate. In addition, 2D gel analysis of the MWF sample resulted in the same profile shown in Fig. 3.

DISCUSSION

Sequence analysis of LMP2 and LMP7 has shown that the homology of the LMP-2 sequence with N-terminal proteasome subunit sequences begins at amino acid 21. In fact, the first 20 amino acids form the first exon of human (24) and mouse (25) *Lmp2*, and the product of this exon has been suggested to be removed by a posttranslational mechanism. Two alternatively spliced *Lmp7* transcripts have also been detected (24). So far, only one transcript has been found for *Lmp2*. We have demonstrated the existence of two forms of LMP2. Based on their 2D gel migration, these probably represent the unprocessed and processed forms of LMP2, although the possibility that they are the products of alternatively spliced transcripts cannot be discounted.

We have demonstrated that the anti-LMP2 serum immunoprecipitates a complex similar to proteasome. This complex lacks LMP7 and has only one of the isoforms of LMP2. Preclearing experiments show that this complex is a distinct complex that contains four unique subunits, including LMP2a, thus suggesting that the LMP2A complex is not a breakdown product of mature proteasomes. Several observations point to the LMP2A complex being an assembly

precursor to the mature proteasome. We do note a reduction in the intensity of the LMP2A complex subunits, which correlates with an increase in some of the subunits of the proteasome profile in the pulse–chase experiments. None of the LMP2A complex subunits increased in intensity. The LMP2A complex only has the LMP-2a subunit, which is probably the precursor of the mature LMP2b subunit. In addition, only one band is seen in Western analysis with anti-LMP2 serum (Fig. 1), and the intensity of LMP2a in Coomassie blue-stained 2D gels of mAb 2-17 immunoprecipitates is significantly lower than that of LMP2b (data not shown), suggesting that the steady-state levels of the LMP2A complex are low. These facts suggest that LMP2A may be assembled into the mature (20S form) proteasome, in which the LMP2 subunit may be inaccessible to our antibody. Processing of all the "unique" subunits of the LMP2A complex could potentially convert it to a form with the subunit pattern of proteasomes. In this regard, cDNA and protein sequencing of many other proteasome subunits have indicated that these subunits can be divided into two groups, one of which has a presequence that is removed (24). These results are summarized in Fig. 6.

The LMP2A complex has been precipitated from ammonium sulfate fractions of HeLa cells (23), and our pulse–chase experiments show that it is present for sufficient periods so as to be physiologically relevant. The 20S form of proteasome is converted to the 26S form by the addition of several subunits. The two forms have different functions. It is conceivable that the LMP2A complex may have a separate enzymatic function, which is modified upon addition of LMP7 and maturation to proteasome.

Several populations of proteasome have been previously reported in mouse (16), human (23), *Drosophila* (26), chicken (27), and rabbit (28). In *Drosophila melanogaster* three forms of 20S proteasome differing in their specific proteolytic activity against fluorogenic substrates and in the enhancement of this activity to SDS treatment have been isolated. Similarly, multiple forms of 20S proteasome and two forms of the 26S proteasome have been identified in rabbit reticulocyte lysates. In both cases, however, no differences in proteasome subunit content were found. In the mouse and human, the different populations do show subunit differences. In addition, the 2D gel pattern of both *Drosophila* (26) and chicken embryonic muscle (27) is altered in a developmentally dependent fashion. It is possible that the proteasome consists of a core to which additional proteins defining specific functional roles are added as required. The addition of LMP2 and LMP7 may represent such a case. In fact, the specificity of enzymatic cleavage of fluorogenic peptide substrates by proteasomes containing or lacking the LMP subunits is markedly different (29, 30). In addition, a level of functional redundancy may exist in the proteasome, with several subunits able to perform the same function. In this regard, all of the known eukaryotic proteasome subunits appear to be encoded by two homologous families of genes, which are likely to have evolved from a common ancestral gene (22). In fact, the archaebacterial *Thermoplasma acidophilum* proteasome is composed solely of two nonidentical subunits, α and β, present in multiple copies.

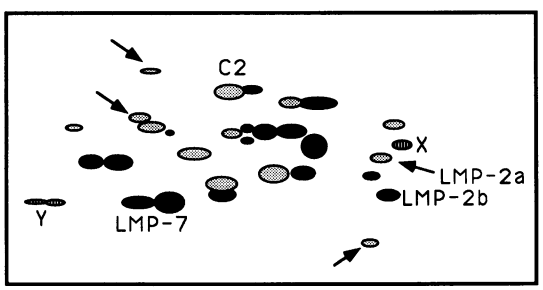

FIG. 6. Diagrammatic representation of the 2D gel profiles of the proteasome complex and the LMP2A complex. Filled spots indicate subunits unique to the proteasome complex. Stippled subunits are those found in the LMP2A complex; unique subunits are indicated by arrows. Subunit X is unique to T1 and T2.

In summary we have identified the human proteasome subunits corresponding to LMP2 and LMP7. LMP2 is in two forms, one of which is unique to a population distinct from a population containing LMP7 and the other isoform of LMP2. We have speculated on the role of the MHC-encoded proteasome subunits. Although this still remains conjectural, a relative comparison of the location and proteolytic activities of proteasome and the LMP2A complex should shed light on the role of the MHC-encoded proteasome proteins.

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