

## High molecular weight antigens present on human T cells

(crossreacting antigens/glycoprotein family/glycosylation processing)

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**ABSTRACT** A series of eight high molecular weight (140,000–220,000) glycoproteins on human peripheral T cells were recognized by radioimmunoprecipitation with a rabbit antiserum. The pattern of antigens present on each of eight human T cell lines studied was unique, and no line displayed the range of antigens present on peripheral T cells. The pattern of bands on peripheral T cells changed after allogeneic or lectin stimulation. Adsorption/elution experiments with antiserum showed that some of these proteins were antigenically related, and at least three different groups of proteins were present. Two of these groups could be partially distinguished by their ability to bind to ricin or lentil lectin and by their reactivity with two additional rabbit antisera. On some cell lines, it was found that proteins bound by lentil lectin but not ricin were precursors of higher molecular weight material recognized by ricin. Taken together, the data suggest that these proteins may be the products of a multigenic or multiallelic system, probably equivalent to the murine Ly 5 antigens.

The pattern of reactivity of a rabbit antiserum (C51) raised against peripheral blood lymphocytes (PBL) from a patient with Sézary syndrome has recently been characterized (in collaboration with R. Edelson, K. Rosenthal, and S. Alexander) and will be published elsewhere. After thorough adsorption on a B lymphoblastoid cell line (from an HLA identical sister of the patient), this antiserum still recognized various antigens on human PBL T cells and T-cell lines. Radioimmunoprecipitation revealed that the most prominent antigens recognized by the antiserum on human PBL T cells consisted of a cluster of proteins with  $M_r$  between 140,000 and 220,000. On the T-cell line CEM, fewer protein bands in a different pattern occurred between  $M_r$  140,000 and 195,000. Examination of non-T lymphoid cell lines revealed that the antiserum reacted weakly with one or two antigens of similar  $M_r$  on some cells, so that not all of these high  $M_r$  antigens could be considered to be T-cell specific. However, they were much more abundant on T cells than on other cell types.

In the present communication, the high  $M_r$  antigens recognized by C51 antiserum on various human T-cell lines, PBL T cells, stimulated PBL T cells, and T-cell growth factor (TCGF)-dependent lines derived from them have been compared. In addition, two rabbit anti-T cell antisera were used which also identified high  $M_r$  T-cell antigens recognized by C51. Importantly, the other antisera, anti-Jan and anti-Doh, appeared to dissect reciprocal sets of high  $M_r$  antigens, all of which were identified by C51 antiserum. Evidence will also be presented that some of the high  $M_r$  antigens are antigenically related.

## MATERIALS AND METHODS

**Cells.** The following human cells were used: T lymphoblastoid lines CEM, HSB, MOLT 4, YT4E, HPB-MLT, TALL-1, and RPMI 8402; Epstein-Barr virus-transformed B lymphoblastoid lines McB, 23B, and 22B; null lines REH and Nalm 1. Normal PBL T cells were isolated from lymphocytes (separated on Ficoll/Hypaque) by passage through a column containing Sepharose conjugated with purified antibody to human Fab. Retained B cells were eluted with 1% IgG, and any contaminating T cells were removed by rosetting with sheep erythrocytes. PBL T cells were stimulated at 6-day intervals by addition of phytohemagglutinin (PHA; Difco) at 10  $\mu\text{g}/\text{ml}$  or irradiated B lymphoblastoid cells and labeled 2–3 days after each stimulation. TCGF-dependent T-cell lines were established through the use of human TCGF (1) from stimulated normal PBL T cells (22T, 23T, McMEn T), leukemic cells [Doh T, Rob T, Hut 102 (2)], and a hypogammaglobulinemic patient (Perk T) and were maintained in cultures containing TCGF. All lines were grown in RPMI-1640 containing 10% fetal calf serum.

**Antisera.** The three antisera used were raised in rabbits against human PBL cells. Anti-Doh and C51 serum were made against leukemic PBL T cells; and anti-Jan was against normal PBL T cells. The cells against which anti-Doh was directed have been described (3). Anti-Jan and C51 antiserum were adsorbed on B lymphoblastoid lines prior to use. However, anti-Doh was used unadsorbed. All three antisera reacted with >95% of PBL T cells and <5% of B cells. In some experiments, adsorbed C51 antiserum was further adsorbed on live REH cells at 4°C. The cells were washed thoroughly and bound antibody was eluted by addition of 0.23 M glycine-HCl (pH 2.8). After 5–10 min at 4°C, cells were centrifuged and the eluted antibody solution was neutralized before storage.

**Labeling and Radioimmunoprecipitation.** Cells were metabolically labeled by growing  $1-2 \times 10^7$  cells in methionine-free RPMI-1640 containing 10% dialyzed fetal calf serum for 6–12 hr in the presence of 0.5 mCi (1 Ci =  $3.7 \times 10^{10}$  becquerels) of [ $^{35}\text{S}$ ]methionine. Surface iodination was carried out on  $2-3 \times 10^7$  washed cells for 15 min by using Iodogen (Pierce) as a catalyst with 0.5 mCi of [ $^{125}\text{I}$ ]NaI. After labeling, washed cells were extracted for 1 hr on ice in 1% Nonidet P-40/2 mM phenylmethylsulfonyl fluoride/10 mM Tris-HCl, pH 7.4. Extracts were centrifuged at  $12,000 \times g$  for 10 min prior to storage at  $-20^\circ\text{C}$  and were recentrifuged upon thawing. For radioimmunoprecipitation (4), labeled extract was allowed to react with normal rabbit serum and then with fixed *Staphylococcus aureus* Cowan I strain (SaCI) to remove material bound

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Abbreviations: PBL, peripheral blood lymphocytes; TCGF, T-cell growth factor; PHA, phytohemagglutinin; SaCI, Cowan I strain of *Staphylococcus aureus*.

nonspecifically. Specific antiserum was then treated with extracts for 60 min; immune complexes were adsorbed onto SaCI and then after 30 min were washed extensively. Immune complexes were dissociated by boiling for 5 min in 50 mM Tris-HCl, pH 6.8/2% NaDodSO<sub>4</sub>/15% glycerol/2% 2-mercaptoethanol. Supernatants were analyzed on 22-cm-long 7.5–15% gradient acrylamide gels containing NaDodSO<sub>4</sub> (5). Radioactive markers (New England Nuclear) were used to calibrate  $M_r$ s. Fluorography with Enhance (New England Nuclear) was carried out on dried gels containing [<sup>35</sup>S]methionine-labeled proteins by using Kodak XRP film at -70°C. Intensifying screens were used to reduce exposure times for <sup>125</sup>I-labeled proteins.

## RESULTS

**T Lymphoblastoid Cell Lines.** The adsorbed C51 serum immunoprecipitated four or five bands of  $M_r$  145,000–250,000 on some T-cell lines (e.g., CEM, TALL-1) (Fig. 1, lanes a and h), whereas on other T-cell lines only one or two bands in this  $M_r$  region could be distinguished (e.g., RPMI 8402, HPB-MLT; Fig. 1, lanes e and f). Of the eight T-cell lines tested, no two presented identical banding patterns, although HPB-ALL and HPB-MLT were quite similar (Fig. 1, lanes f and g). All of the T-cell lines, however, did have a band around  $M_r$  200,000 and, with the exception of RPMI 8402, all expressed at least one additional band between  $M_r$  150,000 and 175,000. Not all lines expressed an equivalent amount of these antigens (e.g., YT4E and RPMI 8402 gave weak bands relative to CEM and HSB). Bands from one cell line often migrated slightly differently from those from other lines, suggesting a multiplicity of protein species. Both [<sup>35</sup>S]methionine and [<sup>125</sup>I]iodine labeling methods gave similar results with most cell lines. Exceptions were CEM and MOLT 4 in which some lower  $M_r$  bands (140,000–165,000) were not iodinated (Figs. 1, lanes a and c, and 2, lanes a and c). In contrast, the lower MW bands of HSB were iodinated, indicating that they were available at the cell surface (Figs. 1, lane b, and 2, lane b).

**Non-T-Cell Lines.** Various lymphoid cell types, in addition to T cells and T-cell lines, express high  $M_r$  antigens recognized by C51 antiserum. Epstein-Barr virus-transformed B-cell lines yielded only two weak high  $M_r$  bands, at 220,000 and 165,000. Nalm 1, a "pre-B null" cell (6), displayed no high  $M_r$  antigens reactive with C51 antiserum. However, a second null cell line, REH, expressed a single prominent broad band at  $M_r$  215,000–235,000 (Fig. 1, lane i).

**Normal PBL T Cells, Thymocytes, and PBL T Cells Stimulated with PHA or Allogeneically.** [<sup>35</sup>S]Methionine-labeled PBL T cells immunoprecipitated with adsorbed C51 antiserum gave a pattern of seven bands straddling  $M_r$  200,000 (165,000–230,000), with an eighth band slightly lower, at  $M_r$  140,000 (Fig. 2, lane d). The most intense bands were a doublet at  $M_r$  195,000, with the higher and lower bands becoming progressively weaker. Surface iodination-labeled fewer bands



FIG. 1. Immunoprecipitations, by C51 antiserum, of [<sup>35</sup>S]methionine-labeled T-cell lines. Lanes: a, CEM; b, HSB; c, MOLT 4; d, YT4E; e, RPMI 8402; f, HPB-MLT; g, HPB-ALL; h, TALL-1; i, REH (weak bands at  $M_r$  195,000 and 140,000 were found in normal serum controls). In all of the figures, gels are aligned at  $M_r$  200,000 but variability in gradients and photographs results in only approximate alignment for the  $M_r$  140,000 band.

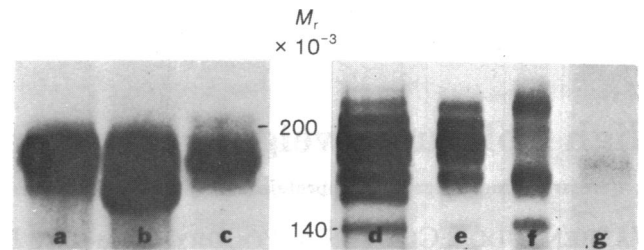


FIG. 2. Immunoprecipitations, by C51 antiserum, of <sup>125</sup>I-labeled T-cell lines and normal cells. Lanes: a, CEM; b, HSB; c, MOLT 4; d, <sup>35</sup>S-labeled PBL T cells; e, PBL T cells; f, PBL B cells; g, thymocytes.

(usually only six rather than eight) (Fig. 2, lane e), which were accurately aligned with the [<sup>35</sup>S]methionine-labeled pattern. Iodinated PBL B cells precipitated with C51 antiserum displayed bands only at  $M_r$  220,000, 165,000, and 140,000 (Fig. 2, lane f). Precipitations with extracts of iodinated human thymocytes yielded a single weak band at  $M_r$  175,000 (Fig. 2, lane g).

Immunoprecipitations with extracts from stimulated T cells showed that the pattern of high  $M_r$  antigens changed with time and depended on the mode of stimulation (Fig. 3). PBL T cells stimulated with PHA for 3 days or 10 days rapidly lost the two bands larger than  $M_r$  200,000 and the bands between 165,000 and 200,000 intensified. After allogeneic stimulation, the bands at 200,000 and 220,000 were initially the strongest but after 10 days the top band disappeared and that at about 180,000 was most prominent. After 3 weeks, the patterns resulting from both types of stimulation were similar, with the heaviest bands at 185,000–200,000 and two weaker ones between 165,000 and 185,000. This pattern resembled that present on the T-cell lines discussed earlier. After both types of stimulation, the lowest band (at 140,000) disappeared very rapidly.

**TCGF-Dependent T-Cell Lines.** The Rob T and Hut 102 lines were derived separately from a patient with a cutaneous T cell lymphoma. Hut 102 is thought to represent malignant cells from a neoplastic lesion (2), whereas Rob T was from normal peripheral T cells. Rob T expressed material of  $M_r$  165,000–200,000, similar to that seen on PHA-stimulated PBL T cells (Fig. 3, lane h). Hut 102 possessed additional material between 200,000 and 225,000 (Fig. 3, lane i). The other line studied with C51 antiserum (Doh T) had a broad band at 200,000 and two sharper bands between 165,000 and 185,000 (not shown). Hence, these TCGF-dependent T-cell lines also resembled T lymphoblastoid cell lines more than PBL T cells.

**Adsorption of C51 Antiserum by Various Cell Lines.** When adsorbed C51 antiserum was further adsorbed with HSB, CEM, or MOLT 4, no reactivity remained against high  $M_r$  bands on

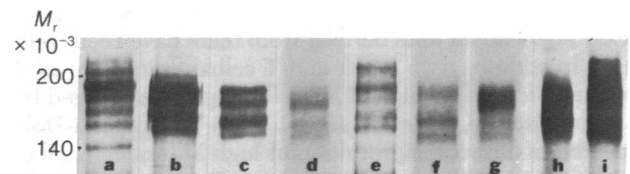


FIG. 3. Immunoprecipitations, by C51 antiserum, of [<sup>35</sup>S]methionine-labeled normal and stimulated T cells and TCGF-dependent T-cell lines. Lanes: a, PBL T cells; b, PBL T cells, ×PHA<sup>1</sup>; c, PBL T cells, ×PHA<sup>2</sup>; d, PBL T cells, ×PHA<sup>3</sup>; e, PBL T cells, ×22B<sup>1</sup>; f, PBL T cells, ×22B<sup>3</sup>; g, PBL T cells, ×22B<sup>5</sup>; h, Rob T; i, Hut 102. Super-script number refers to the number of times the cells were stimulated prior to labeling. 22B is a B-cell line used for allogeneic stimulation.

the other two lines (Fig. 4, lanes a and b). After YT4E was used for a similar adsorption, the band at 140,000 could still be precipitated from [<sup>35</sup>S]methionine-labeled CEM, although all reactivity against the higher  $M_r$  bands was abolished (Fig. 4, lane c). The fact that the pattern of C51 antiserum reactive high  $M_r$  antigens was different for each line used in these adsorptions suggested that some of the antigens might represent a family of antigenically related molecules. To explore this idea, adsorbed C51 antiserum was further adsorbed on live REH, a cell line chosen because it expressed but a single, broad band around  $M_r$  225,000 (Fig. 1, lane i). The cells were washed, and bound antibody was eluted with a low pH buffer. After neutralization, this eluted antibody was used to carry out precipitations of other cells. When tested on MOLT 4 (Fig. 4, lane d), the antibody eluted from REH precipitated the multiplicity of bands seen with the C51 antiserum (Fig. 1, lane c). Thus, those antibodies that recognized antigens of a restricted  $M_r$  range on the null cell REH recognized a much broader range of antigens on the T cell MOLT 4.

With iodinated PBL T cells as the target, C51 antiserum eluted from REH precipitated only the top four of the upper cluster of five bands. It was unreactive with the bands below  $M_r$  about 170,000 (Fig. 4, lanes e and f). On iodinated PBL B cells, C51 antiserum eluted from REH precipitated the 220,000 band (not shown). When extract from PHA-stimulated PBL T cells (Fig. 4, lane g) was precipitated with adsorbed C51 antiserum further adsorbed with CEM, only the two lower bands ( $M_r$  155,000–175,000) of the four normally present were seen (Fig. 4, lane h). These and results of other similar experiments suggested that there is a "family" of proteins of  $M_r$  between 175,000 and 230,000 which share antigenic determinants but that some antigens of  $M_r$  170,000 and lower do not belong to this group.

**Lectin Binding of Antigens Recognized by C51 Antiserum.** Among the CEM antigens precipitated by C51 the broad band at  $M_r$  175,000–195,000 bound strongly to lectins from ricin and wheat germ but only weakly to lentil lectin (Fig. 5). The bands at 140,000 and 155,000–165,000, however, bound only to lentil lectin. Similarly, on HSB, the heavy band at 160,000 bound to lentil lectin and the weaker larger bands bound to ricin lectin. On REH the single high  $M_r$  band ( $\approx$ 220,000) bound to all three lectins. Antigens on PBL T cells and PBL T cells stimulated either with PHA or allogeneically were examined with lectins from lentil and ricin. The band at 140,000 in normal PBL T cells that disappeared upon stimulation bound to both lentil and ricin lectins with equal avidity. The highest  $M_r$  bands seen ( $\approx$ 220,000 on allogeneically stimulated cells) also seemed to be bound by both ricin and lentil lectins with equal avidity. Apart from these bands, the higher  $M_r$  bands bound to ricin more readily than to lentil lectin, whereas the reverse was true of the lower  $M_r$  bands.

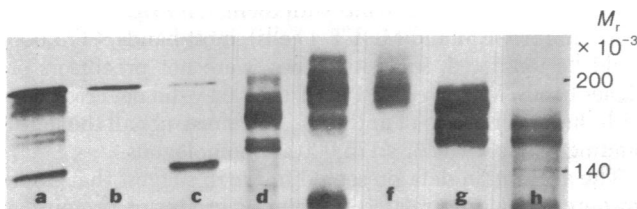


FIG. 4. Immunoprecipitations of T-cell lines and of normal and stimulated peripheral T cells by C51 antiserum adsorbed in various ways. Lanes: a, CEM  $\times$  C51; b, CEM  $\times$  C51, with HSB; c, CEM  $\times$  C51, with YT4E; d, MOLT 4  $\times$  C51, eluted from REH; e, PBL T  $\times$  C51; f, [<sup>125</sup>I]-PBL T  $\times$  C51, eluted from REH; g, PBL T cells  $\times$  PHA<sup>2</sup>  $\times$  C51; h, PBL T cells  $\times$  PHA<sup>2</sup>  $\times$  C51, with CEM. Superscript refers to the number of times the cells were stimulated prior to labeling. Label was <sup>35</sup>S in a, b, c, d, g, and h and <sup>125</sup>I in e and f.

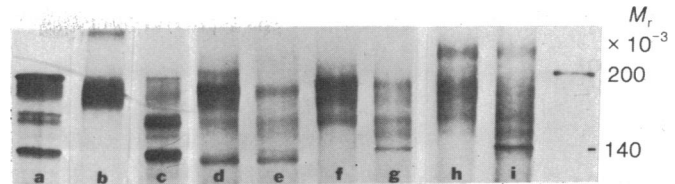


FIG. 5. Lectin binding of various proteins immunoprecipitated by C51 antiserum. Aliquots of lectin conjugated to Sepharose beads were incubated for 1 hr at 4°C with extracts of [<sup>35</sup>S]methionine-labeled cells, washed, and then eluted with the appropriate sugar. Immunoprecipitations were then carried out. Lanes: a, CEM; b, CEM, ricin binding; c, CEM, lentil binding; d, PBL T cells, ricin binding; e, PBL T cells, lentil binding; f, PBL T cells  $\times$  PHA<sup>2</sup>, ricin binding; g, PBL T cells  $\times$  PHA<sup>2</sup>, lentil binding; h, PBL T cells  $\times$  23B<sup>1</sup>, ricin binding; i, PBL T cells  $\times$  23B<sup>1</sup>, lentil binding. Superscript refers to the number of times the cells were stimulated prior to labeling. 23B is a B-cell line used for allospecific stimulation.

**Heteroantisera Against Other Human T Cells.** The other two antisera used (anti-Jan and anti-Doh) recognized antigens also recognized by C51 antiserum in the high  $M_r$  region. However, each reacted with an essentially complementary subset of high  $M_r$  antigens. Anti-Jan (Fig. 6, lane c) reacted mainly with the larger ( $>$ 180,000) proteins recognized by C51 antiserum on PBL T cells (Fig. 6, lane b), whereas anti-Doh reacted only weakly with those  $>$ 200,000 and most strongly with those at 160,000–175,000 (Fig. 6, lane a). Neither anti-Jan nor anti-Doh reacted with the 140,000 band on PBL T cells. This pattern of reactivity of these two antisera applied to PHA-stimulated PBL T cells (Fig. 6, lanes d–f) and to the TCGF-dependent T-cell lines as well (Fig. 6, lanes g–j) and was reminiscent of the lectin binding results (Fig. 5, lanes d–g). Ricin lectin (like anti-Jan) bound more strongly to the higher  $M_r$  bands, and lentil lectin (like anti-Doh) bound more strongly to the lower bands. Some degree of crossreactivity was apparent, particularly in the 170,000–180,000 region with both lectins and antisera.

Anti-Doh antiserum was used to carry out immunoprecipitations on a number of TCGF-dependent T-cell lines and a smaller number of syngeneic Epstein-Barr virus-transformed B-cell lines. Very little material was precipitated from the B-cell line in the region 140,000–230,000 compared to a syngeneic T cell line (Fig. 6, lanes k and l). On most TCGF-dependent T-cell lines (22T, 23T, Per T, Hut 102, McMen T) anti-Doh precipitated doublets between 175,000–195,000 and 155,000–165,000 (Fig. 6, lanes i, l, and m). As a group, the high  $M_r$  antigens recognized by anti-Doh antiserum on TCGF-dependent T-cell lines resembled each other more closely than did T lymphoblastoid lines precipitated with C51 antiserum.

**Pulse-Chase and Tunicamycin Treatment of CEM Cells.** Were some of the high  $M_r$  bands recognized by C51 antiserum on the T-cell lines intermediates in the production of more heavily glycosylated bands? The T-cell line CEM was chosen for study of this question because it contained bands at  $M_r$  155,000–165,000 which were detected by [<sup>35</sup>S]methionine labeling but not by surface radioiodination, suggesting that they could be intracellular precursors of the  $M_r$  175,000–195,000 bands which were labeled by both [<sup>35</sup>S]methionine and <sup>125</sup>I. Moreover, the lower  $M_r$  bands in this cell line were bound to lentil lectin but not to ricin. This is consistent with their containing high-mannose oligosaccharides which lack the galactose required for ricin binding. The higher  $M_r$  bands were bound by ricin, suggesting that they contained complex glycans.

In pulse-chase experiments, the pulse of [<sup>35</sup>S]methionine was rapidly incorporated into the doublet at 155,000–165,000, reaching a maximum at 15 or 30 min (Fig. 7, lanes a and b). A chase with unlabeled methionine was begun at 30 min. By 60

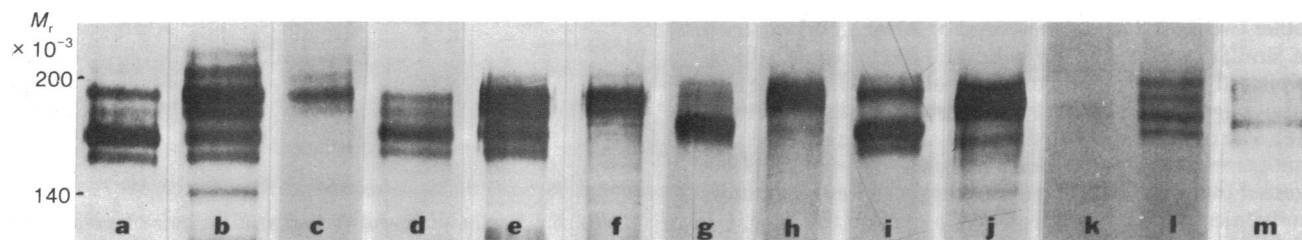


FIG. 6. Immunoprecipitations by C51, anti-Doh, and anti-Jan antisera of [ $^{35}$ S]methionine-labeled peripheral T cells and various cell lines. Lanes: a, PBL T  $\times$  anti-Doh; b, PBL T  $\times$  C51; c, PBL T  $\times$  anti-Jan; d, PBL T  $\times$  PHA  $\times$  anti-Doh; e, PBL T  $\times$  PHA  $\times$  C51; f, PBL T  $\times$  PHA  $\times$  anti-Jan; g, Rob T  $\times$  anti-Doh; h, Rob T  $\times$  anti-Jan; i, Hut 102  $\times$  anti-Doh; j, Hut 102  $\times$  anti-Jan; k, 23B  $\times$  anti-Doh; l, 23T  $\times$  anti-Doh; m, McMEn T  $\times$  anti-Doh.

min, radioactivity began to appear in the doublet at 175,000–195,000 and was more intense at 120 min. By 9 hr the doublet at 155,000–165,000 had disappeared and all material had been converted to the doublet at 175,000–195,000 (Fig. 7, lanes c, d, and e). The lentil lectin binding band at  $M_r$  140,000 was labeled more slowly and did not seem related to the series of higher  $M_r$  bands.

Experiments performed with tunicamycin to inhibit *N*-linked glycosylation showed a prominent component on CEM cells at  $M_r$  145,000–150,000 (Fig. 7, lane f). This is smaller than the doublet labeled in the shortest pulse with [ $^{35}$ S]methionine at 15 min (Fig. 7, lane a). The small amounts of material at higher  $M_r$ s were presumably due to incomplete inhibition of glycosylation under the conditions of the experiment. Thus, the nonglycosylated protein has an apparent  $M_r$  of about 150,000 and can be processed to higher  $M_r$  species. The fact that doublets occur at both 155,000–165,000 and 175,000–195,000 suggests the possibility that more than one protein species could be involved or that the processing is extremely complex.

## DISCUSSION

A group of high  $M_r$  antigens present on human T cells that are recognized by various antisera have been studied by radioimmunoprecipitation followed by NaDodSO<sub>4</sub>/polyacrylamide gel electrophoresis. T cells express some of these antigens at  $M_r$ s where no bands occur on PBL B cells (180,000–215,000). However, these bands share antigenic determinants with a  $M_r$  220,000 band on PBL B cells, so it is uncertain which, if any, of these is T-cell specific. Normal human PBL T cells expressed a greater range of these antigens than any of the transformed T lymphoblastoid cell lines examined. Do PBL T cells express so many bands because they constitute a diverse population containing several subsets of cells, each possessing a subset of the bands seen in the immunoprecipitations? After stimulation by PHA or allogeneically, both the pattern and number of bands recognized by C51 antiserum clearly changed. After a

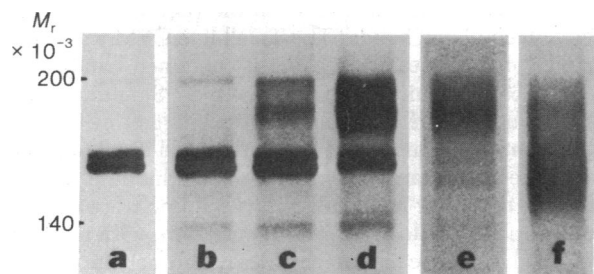


FIG. 7. Pulse-chase and tunicamycin-treated [ $^{35}$ S]methionine-labeled CEM cells immunoprecipitated with C51 antiserum. Lanes: a, CEM, 15-min label; b, CEM, 30-min label; c, CEM, 30-min label + 30-min chase; d, CEM, 30-min label + 120-min chase; e, CEM, 30-min label + 9-hr chase; f, CEM, tunicamycin-treated (1  $\mu$ g/ml, 90 min) prior to labeling for 9 hr.

few days (corresponding to the time of maximum blastogenesis) the allogeneically and PHA-stimulated cells had different band patterns. However, at later times, corresponding to morphological reversion to small lymphocytes, both types of stimulation produced a similar pattern.

The distinctiveness of banding patterns in each of the TCGF-dependent cell lines is interesting in view of the apparently similar selection process involved in the establishment of these lines. In culture with TCGF, >80% of PBL T cells die (1); the surviving, dividing population of lymphocytes is derived from a small subpopulation. The diversity of high  $M_r$  antigens recognized by various antisera on different TCGF-dependent lines suggests that these lines are not necessarily derived from the same T-cell subpopulation. Moreover, the diversity of the high  $M_r$  bands found on transformed T lymphoblastoid lines suggest that there is no simple relationship between subsets and patterns of high  $M_r$  antigens. All eight autonomous T cell lines examined were different with respect to these antigens (although some were similar). At present, insufficient data are available to relate banding patterns of these antigens to normal T-cell subsets, but the diversity of antigens and of banding patterns suggests the possibility that they are important in the specific functions of T cells. Many of these high  $M_r$  antigens shared antigenic determinants—e.g., the bands between 180,000 and 230,000 present on PBL T cells, all of the bands on MOLT 4 cells, and at least the higher  $M_r$  bands (>180,000) on other lines tested. It is likely that all the bands immunoprecipitated on T cells and T-cell lines above 170,000 share antigenic determinants with each other, with the 220,000 band on PBL B cells, and with the 225,000 band on the null cell line REH. Whether the lower  $M_r$  bands (140,000–170,000) form another crossreacting group was not determined. At least some bands in that smaller size range (e.g., on MOLT 4) crossreacted with the upper group.

Because MOLT 4 resembled CEM in the pattern of high  $M_r$  bands expressed, it is likely that the 150,000 bands on MOLT 4 represented precursors of the higher  $M_r$  bands and therefore shared antigenic determinants with them. On PBL T cells and HSB (unlike CEM and MOLT 4 cells), most bands <175,000 could be iodinated, suggesting they were not precursors of higher bands. Moreover, on PBL T cells, they did not crossreact with the upper group of antigens. Therefore, not all the lentil binding bands with  $M_r$  <175,000 are homologous.

The adsorption data presented in Fig. 5 suggest that three immunologically unrelated proteins or groups of proteins—140,000,  $\approx$ 170,000, and 180,000–230,000 (and lower precursor bands on CEM and MOLT 4)—occur. Results with anti-Doh and anti-Jan reinforced this conclusion. The 170,000 band prominently recognized by anti-Doh on PBL T cells did not share antigenic determinants with the 180,000–220,000 group of proteins (Figs. 4, lanes e and f, and 6, lane a); anti-Jan reacted with those. Anti-Jan also tended to react more strongly with

ricin binding proteins and anti-Doh reacted with those proteins that bound lentil lectin. Lectin binding analyses revealed that all the high  $M_r$  antigens recognized by C51 antiserum were glycoproteins; hence, the  $M_r$ s assigned to them are only approximate.

High  $M_r$  glycoproteins on human lymphocytes have been observed previously, but antisera have been little used in their analysis. Andersson *et al.* (7) labeled several high  $M_r$  glycoproteins on human lymphoid cells and cell lines by the sodium boro[ $^3\text{H}$ ]hydride method; however, their labeling and gel systems were less sensitive and fewer bands were detected or resolved. The four protein bands they observed (160,000, 165,000, 180,000, and 200,000) on PBL T cells were more intense after PHA stimulation but were only slightly changed in allogeneically stimulated T cells (8). They suggested that these four bands are T-cell specific because they reacted with an anti-T cell antiserum (8). However, the proteins described here in that  $M_r$  region share determinants with B and null cell proteins. Recently, Niaudet and Greaves (9) made an anti-Sézary syndrome antiserum that precipitated a protein of  $\approx 170,000$  from several T-cell lines, and Anderson and Metzgar (10) prepared an antiserum against HSB cells which detected a lentil lectin-binding glycoprotein of  $M_r \approx 170,000$  on HSB, PBL T cells, and thymocytes (11), almost certainly the same as proteins of that size seen on these cells with C51 antiserum.

On murine lymphoid cells, several high  $M_r$  components have been described, and changes in patterns of these antigens have been seen in alloantigen-activated T cells (12). Recently, Sarmiento *et al.* (13) described surface proteins of cloned murine T-cell lines with either helper or cytotoxic activity. Differences in the  $M_r$  160,000–220,000 region distinguished the various T-cell clones they studied. The patterns of multiple bands seen closely resemble banding patterns seen here on human T cells and lines. The authors commented that monoclonal antibodies directed against polypeptides in this region distinguished helper T cell clones from cytotoxic T cell clones. Omary *et al.* (14) and Michaelson *et al.* (15) recently demonstrated that antisera directed against the murine Ly 5 antigen precipitated several components of  $M_r$  between 175,000 and 220,000. A monoclonal antibody directed against a mouse T cell lymphoma  $M_r$  200,000 protein apparently recognized the same protein as an anti-Ly 5.1 antiserum (14). It seems likely that the antigens described here represent human equivalents of murine T200 and related proteins and, by extension, of the murine Ly 5 system. The Ly 5 antigens were originally thought to be T-cell specific but have recently been found on most or all lymphoid cell types (15). The anti-Ly 5 antisera react with multiple bands of  $M_r$  175,000, 185,000, and 220,000 on murine splenocytes but with only a single broad band of  $M_r$  175,000 on thymocytes (15), similar to data for human cells presented here. The  $M_r$  220,000 antigen is the dominant antigen on murine B cells, also in agreement with the present findings.

The complexity of the antigens described here suggests that they may be the products of a multigenic or multiallelic system. The observation that antiserum adsorbed on and eluted from REH cells crossreacts with proteins of much lower apparent  $M_r$  on other cell lines is striking, as are the differences in band positions in different cell types. Some of the differences observed could be the consequence of cell-specific glycosylation reactions. The pulse-chase experiment confirms that processing

by glycosylation may account for some of the diversity of bands. However, the fact that some of the lower  $M_r$  bands can be labeled by iodination and therefore are expressed at the surface (e.g., on HSB cells) suggests that some of the variability is due to a phenomenon other than glycosylation precursors. The occurrence of alleles of the Ly 5 antigen in the mouse supports the idea that some of these human proteins may be products of polymorphic genes. Recently, several alloantisera have been described (16, 17) which detect T-cell polymorphisms in man and which could recognize these proteins. Insight into the significance of the heterogeneity of the high molecular weight glycoproteins may be gained when the chemical basis of the heterogeneity is more thoroughly understood. In particular, the complexity of the present data suggests that the variability observed could be based on polypeptide variations (as in the HLA system, where the structural genes are polymorphic) or glycosylation differences (as in the ABO system, where the genes for glycosylation enzymes are polymorphic) or both.

**Note Added in Proof.** Dunlap *et al.* (12) have shown that the proteins of  $M_r$  200,000 and 187,000 on murine T cells have identical tryptic peptide maps and that the protein of  $M_r$  170,000 is distinct (18). Their data are complementary to those presented here.

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