Botany. In the article “Isolation, sequence, and bacterial expression of a cDNA for (S)-tetrahydroberberine oxidase from cultured berberine-producing Coptis japonica cells” by Naosuke Okada, Nozomu Koizumi, Toshihiro Tanaka, Hiroaki Okubo, Shigetada Nakanishi, and Yasuyuki Yamada, which appeared in number 2, January 1989, of Proc. Natl. Acad. Sci. USA (86, 534–538), the authors request that the following correction be noted. The amino acid sequence of part of a 28-kDa protein previously reported to copurify with (S)-tetrahydroberberine oxidase activity was determined, and this sequence was used to design oligonucleotides that were, in turn, used to isolate clones from a cDNA library. Upon further investigation, we found that the “purified” (S)-tetrahydroberberine oxidase was heavily contaminated with triosephosphate isomerase (EC 5.3.1.1) and that the sequence determined was actually that of C. japonica triosephosphate isomerase. Additionally, the DNA sequence of the cDNA clone pTHB201 (p. 536) encodes C. japonica triosephosphate isomerase rather than C. japonica (S)-tetrahydroberberine oxidase, and the RNA hybridization analysis (p. 535) detected triosephosphate isomerase mRNA. Data to support this correction has been presented elsewhere (1).


Medical Sciences. In the article “Point mutations define positions in HLA-DR3 molecules that affect antigen presentation” by Elizabeth Mellins, Benjamin Arp, Devinder Singh, Beatriz Carreno, Laura Smith, Armead H. Johnson, and Donald Pious, which appeared in number 12, June 1990, of Proc. Natl. Acad. Sci. USA (87, 4785–4789), the authors wish that the following corrections be noted. On page 4785, in line 8 under Materials and Methods, DRB3*0301 should be DRB3*0101. On page 4789, ref. 23 should be as follows:


Immunology. With regard to the article “Secretion and cell surface expression of IgG1 are impaired in human B lymphoblasts that lack HLA-A, -B, and -C antigens” by William J. Burlingham, Stephanie S. Ceman, and Robert DeMars, which appeared in number 20, October 1989, of Proc. Natl. Acad. Sci. USA (86, 8005–8009), the authors request that the following correction and retraction be noted. In Fig. 1, SDS/polyacrylamide gel electrophoresis was used to show that surface IgG(κ) was absent from the HLA-A, -B, -C “null” B-LCL mutant 721.221 (lane f) and the pHeBo vector-alone control transfent (lane g) and was greatly diminished in the pHPT32 vector-alone control transfent (lane j). In contrast, transfent cell lines that expressed transgene-encoded HLA-A1 (lane i), -A2 (lane h), -B5 (data not shown), -B8 (lane 1), or -C (lane k) also expressed membrane and secretory IgG. In addition, the amount of IgG secreted into the cell culture medium was undetectable or greatly diminished in .221 and the two vector-only control transfents in comparison to transfents expressing HLA-A, -B, or -C (Tables 2 and 3). The electrophoretic and secreted IgG observations were highly reproducible. Subsequent pulse-chase and nuclear run-off experiments by S.S.C. showed that the Ig γ chain was not made and that the Ig gene was not transcribed in .221. These observations suggested the conclusion proposed in our publication, which was that expression of HLA-A, -B, or -C was needed for expression of the Ig γ gene. However, while data from one experiment that challenged this interpretation were inadvertently over-looked by the first author, the unusual nature of the findings did prompt continued analysis with regard to other possible explanations of the loss of Ig γ expression. Subsequent work by S.S.C. now indicates that the proposed interpretation is erroneous. Abundant IgG was observed in newly thawed .221 cells that had been cryopreserved soon after isolation of the mutant. This suggested the possibility that expression of the Ig γ chain might have been lost subsequently for a reason unconnected with the loss of HLA-A, -B, and -C expression during the many doublings that preceded use of .221 in the described experiments. Indeed, newly performed transfents of the HLA-A2 and -B8 genes into IgG-.221 did not restore IgG expression. Southern blotting analysis of BamHI-cut DNA with a probe for the Ig γ-gene constant region showed that six bands were present in the parental cell line LCL 721 and in the IgG- early isolate of .221 but that two of the bands were absent in the IgG- version of .221 that was used for our publication. Therefore, we now believe that spontaneous deletion of DNA of the functional Ig γ gene occurred during long-term propagation of mutant .221. One might imagine that the transfents used for our publication were made at a time when the .221 population was a mixture of IgG+ and IgG- cells and that, by remarkable coincidence, all of the transfents expressing HLA-A, -B, or -C were derived from IgG+ cells and the vector-alone controls from IgG- cells. According to this interpretation the vector-alone expressing transfents should express as little Ig γ as .221 itself—i.e., none. The presence of some Ig γ chain (some of abnormal size) in the pHePT32 vector-alone control transfent (Fig. 1, lane j, and Table 3) suggests the possibility that more than one kind of spontaneous event altering Ig γ expression had occurred during production of the transfent cell lines used for our publication. We apologize for the mistake in interpretation and for any inconvenience our report may have caused.
Secretion and cell surface expression of IgG1 are impaired in human B lymphoblasts that lack HLA-A, -B, and -C antigens

(Received 14 April 1989)

ABSTRACT B-lymphoblastoid cell line (LCL) 721.221 lacks HLA-A, -B, and -C class I antigens and transcripts as a result of γ-ray-induced mutations. LCL 721, from which mutant .221 was derived, produces membrane and secreted forms of IgG1(α). In contrast, IgG expression in .221 had these characteristics: (i) γ1 heavy chains were diminished by 98% but were detectable with chain-specific antibodies in cell lysates; (ii) κ light chains were present at normal levels in cell lysates and free κ chains were secreted; (iii) cell-surface-associated IgG and secreted IgG were absent. Mutants that had partially reduced amounts of class I antigens continued to secrete IgG, however, both the absolute amount of IgG secreted and the relative amount of κ vs. intact IgG secreted were abnormal in such partially class I-deficient cells. The failure to export IgG and the deficiency of HLA-A, -B, and -C were not merely coincidental in mutant .221, since production of IgG was restored by transferring a functional HLA-A, -B, or -C gene into .221. Cell surface antigen expression of cloned HLA-A, -B, and -C transgenes introduced into .221 was comparable to that of the same genes in their normal chromosomal locations. These observations reveal a relation between production of HLA class I gene products and production of IgG.

Human class I histocompatibility (HLA) antigens are formed by noncovalent association between a polymorphic 44-kDa α-chain and nonpolymorphic 12-kDa β2-microglobulin. Class I molecules are expressed on a wide variety of cell types including lymphoid, epithelial, and mesenchymal tissue. The best known members of the HLA class I gene family are the HLA-A, -B, and -C loci, which are on the short arm of chromosome 6 and account for >95% of the class I antigen expression on human lymphocytes (1).

Class I incompatibility remains a principal obstacle to clinical organ and bone marrow transplantation. Allelic differences between class I antigens of recipients and donors and tissues result in vigorous allograft rejection (2). Rejection that sometimes occurs in transplantation between HLA-identical siblings may also be mediated by presentation of "minor" histocompatibility antigens by class I antigens of the donor (3). In analogous fashion, class I antigens function in immune responses to intracellular pathogens, especially viruses, by presenting viral antigens to immune-competent T cells (4). These functions of class I antigens in immune reactions require their binding to peptides and subsequent interaction with the T-cell receptor and, in some cases, with the CD8 molecule on CD8+ cytolytic T lymphocytes (5–9).

There is evidence for the involvement of class I antigens in other functions that are related to (10–13) or that may be unrelated to (14, 15) immunity. We describe here a relation between class I gene expression and the production of immunoglobulin in human B lymphocytes. These observations were made with the Epstein–Barr virus-transformed B-lymphoblastoid cell line (LCL) 721 and a collection of its mutant derivatives that had diverse reductions in class I antigen expression. Specifically, IgG1(α) is incorporated into the surface membrane and secreted by LCL 721. Mutant .221 was derived from LCL 721 and lacks HLA-A, -B, and -C antigens because of γ-ray-induced mutations (16). .221 produces undiminished amounts of κ chains and secretes free κ chains into the medium. In striking contrast, the production, cell surface display, and secretion of γ chains, whether free or κ-associated, is only 2–3% or less of that observed in LCL 721. This impairment in γ-chain production and IgG expression was reversed by transferring functional HLA-A, -B, and -C genes into .221, thus implicating normal HLA-A, -B, -C expression directly or indirectly in the production of immunoglobulin.

EXPERIMENTAL PROCEDURES

Cells. The origin of human LCL 721 and basic aspects of γ-ray mutagenesis and isolation of HLA antigen-loss mutants have been described (17). Two groups of cell lines derived from LCL 721 were used in this study. One group consisted of mutants that had either one haplotype deleted (.45) or that had homozygous mutations of at least one class I gene as a result of subsequent mutagenesis of single-haplotype cells (.53, .144, .184, .221). The second category consisted of transfert cells prepared by transferring cloned functional class I genes into the HLA-A, -B, -C-null LCL mutant .221 by means of electroporation. The preparation of the recombinant DNA plasmids and procedures for transferring them and isolating transfents have been described (18). The HLA-A, -B, -C phenotypes of the cell lines used in this study are listed in Table 1.

Cell Culture. Cells were maintained in medium consisting of 85 volumes of RPMI 1640 containing 25 mM Hepes, streptomycin (100 μg/ml), and penicillin (1000 units/ml), supplemented with 15 volumes of a 1:1 mixture of fetal bovine serum and defined, supplemented calf serum (HyClone/Sterile Systems, Logan, UT). The medium was supplemented either with hygromycin (300 μg/ml; Calbiochem) or with 1 μM azaaserine plus 100 μM hypoxanthine as appropriate for the maintenance of transfents (17). In some experiments, the cultures were maintained for 1–3 weeks in medium supplemented with cyprochlorazine hydrochloride (10 μg/ml) (22) as an extra precaution against mycoplasma contamination. The experimental results were the same whether or not this precaution was taken and all cultures

Abbreviations: LCL, B-lymphoblastoid cell line; mAb, monoclonal antibody; γm and γn, membrane and secretory forms of IgG heavy chain.

†To whom reprint requests should be addressed.
Table 1. Human LCL class I antigen-loss mutants and class I gene transfers used in this study

<table>
<thead>
<tr>
<th>LCL</th>
<th>HLA-A, -B, -C* phenotypes</th>
<th>Ref(s).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>721</td>
<td>A1,B8+,Cp/A2,B5,Cm</td>
<td>16</td>
</tr>
<tr>
<td>Mutants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.45†</td>
<td>A2,B5,Cm</td>
<td>16</td>
</tr>
<tr>
<td>.144</td>
<td>A1,B5,Cm</td>
<td>18</td>
</tr>
<tr>
<td>.53</td>
<td>A2,B5,Cm</td>
<td>17</td>
</tr>
<tr>
<td>.184</td>
<td>A1,B5,Cm</td>
<td>19</td>
</tr>
<tr>
<td>.221</td>
<td>A2,B5,C0</td>
<td>20, 21</td>
</tr>
<tr>
<td>Transferrats†</td>
<td></td>
<td>20, 21</td>
</tr>
<tr>
<td>pHBeO→.221</td>
<td>A1,B9,C0</td>
<td></td>
</tr>
<tr>
<td>pHBeO(A1)→.221</td>
<td>A1,B9,C0</td>
<td></td>
</tr>
<tr>
<td>pHBeO(A2)→.221</td>
<td>A2,B9,C0</td>
<td></td>
</tr>
<tr>
<td>pHPT→.221</td>
<td>A1,B9,C0</td>
<td></td>
</tr>
<tr>
<td>pHPT(B5)→.221</td>
<td>A1,B5,C0</td>
<td></td>
</tr>
<tr>
<td>pHPT(B8)→.221</td>
<td>A1,B8,C0</td>
<td></td>
</tr>
<tr>
<td>pHPT(C)→.221</td>
<td>A1,B9,C0</td>
<td></td>
</tr>
</tbody>
</table>

*HLA-C typing with conventional antisera has failed to consistently reveal the C phenotype of LCL 721; therefore, the alleles of C are designated p (paternal) or m (maternal) corresponding to their haplotype.
†Mutant .45 has just one copy of the major histocompatibility complex. The other mutants were derived from .45 or (in the case of .53) from the similar single-haplotype mutant .19.
‡Transferrants are designated as follows: vector(transgene)→recipient cells.

Table 2. Secretion of IgG1 and free κ by HLA class I-loss mutants and by parent LCL 721

<table>
<thead>
<tr>
<th>Protein</th>
<th>ng per 10^6 cells per day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>721</td>
</tr>
<tr>
<td>IgG</td>
<td>1329 (0.04)</td>
</tr>
<tr>
<td>κ</td>
<td>1629 (0.35)</td>
</tr>
<tr>
<td>κ/IgG ratio</td>
<td>1.22</td>
</tr>
</tbody>
</table>

*Mean of triplicate well determinations of supernatants from 24-hr cultures. Numbers in parentheses indicate the standard deviation (SD) as a fraction of the mean.

RESULTS

Impairment of IgG1 Secretion in the HLA-A*BP0C0 Mutant .221. The first indication of impaired IgG production in mutant .221 came from analysis of IgG secretion. In six separate ELISA experiments we were unable to detect IgG in supernatants from cultures after either 24 hr or 7 days of incubation. An example of one such experiment is shown in Table 2. To determine the nature of the lesion and its relation to loss of HLA-A, -B, -C expression, we compared secretion of intact IgG1 and of κ (both IgG-bound and free) by mutant .221 with that by its diploid parent LCL 721, the haplotype-
loss mutant (.45), and mutants lacking expression of HLA-B (.53), HLA-A (.144) and HLA-A and -B (.184). As shown in Table 2, LCL 721 secreted 1.3 μg of IgG per 10^6 cells per day. Loss of the A1, B8 haplotype in mutant .45 was associated with a loss of >50% of IgG secretion; this difference from LCL 721 was reproduced in four separate experiments (data not shown). Loss of HLA-B (.53), but not of HLA-A (.144), resulted in a further 60% reduction (vs. mutant .45) in IgG secretion. Mutant .184, the immediate ancestor of .221, does not express HLA-A and -B antigens but does express HLA-C (Table 1). Although IgG secretion was not reduced compared to .45 or .144, mutant .184 secreted nearly twice as much as HLA-A and -B SECRETANTS in LCL 721, and more than 3 times as much as HLA-B (.45 and .144). The high level of secretion characteristic of mutant .184 was also seen in its derivative HLA-A, -B, -C null mutant .221; however, intact IgG was not detected in culture supernatants of .221 (Table 1). Attempts to detect secretion of IgG or free γ chain by .221, using an Fc-specific coating antibody in the ELISA, gave similar negative results (not shown).

HLA-A, -B, or -C Gene Transfer Restores IgG1 Secretion in Mutant .221. To determine whether impaired IgG1 secretion and HLA class I deficiency were merely coincidental in mutant .221, we analyzed supernatants from 24-hr cultures of HLA-A, -B, or -C gene transfere nts of .221, as well as control transfectants containing only the plasmid vectors, in five separate experiments. The results of a representative experiment (Table 3) indicate that the restoration of normal HLA-A, -B, or -C antigen expression in mutant .221 by gene transfer substantially restored IgG secretion (0.5–1.0 μg per 10^6 cells per day). The pHeBo vector control transfected failure to secrete detectable intact IgG, although a barely detectable amount of free γ chain secretion (6 ng per 10^6 cells per day) was observed with an Fc-specific coating antibody in two separate ELISA experiments (data not shown). In contrast, the pHPT32 vector control transfected reproducibly secreted a small but significant amount of intact IgG, 10–15% of that secreted by HLA-A1, -A2, -C, and -B8 transfere nts. Interestingly, the pHPT vector transfected secreted substantially less κ chain than mutant .221 (Tables 2 and 3).

Lack of Immunoprecipitable Cell Surface-Associated Membrane (γm) and Secretory (γs) Forms of IgG in Mutant .221. We next analyzed surface expression of IgG by surface radioiodination and immunoprecipitation to determine which molecular forms are normally expressed by IgG-secreting LCLs and which (if any) are expressed by the nonsecreting mutant .221. One-dimensional SDS/PAGE of reduced protein A immunoprecipitates of surface-125I-labeled LCL 721 showed both a membrane (γm, 61 kDa) and a secretory (γs, 52 kDa) form of IgG1 heavy chain as well as κ (26 kDa) light chain (Fig. 1, lane a). The coexistence of secretory and integral membrane forms of IgG at the surface of immunoglobulin-secreting B cells has been correlated with the presence of polar immunoglobulin secretory vesicles on the cell surface (29, 30). The presence of such vesicles on LCL 721 was confirmed by immunofluorescence microscopy (D. Hultet, W.J.B., and R.D., unpublished observations). Mutant .45 (lane b) expressed an equal amount of γm but little of the γs form as compared with LCL 721. The HLA-A-loss, -B-loss, and -A, -B-loss mutants (lanes c–e; mutants .144, .53, and .184, respectively) all strongly expressed the γs form, along with a variable amount of γm surface IgG. Mutant .221 (lane f), on the other hand, lacked both γm and γs forms of protein A-precipitable surface IgG. Similar results were obtained in three separate experiments using either protein A or mAb 1410 as immunoprecipitation reagents (mAb 1410 data not shown).

Cell surface expression of IgG was not readily apparent in the pHeBo vector control transfient LCL (Fig. 1, lane g), but in several other experiments, we were able to detect very faint heavy-chain-sized bands with little or no associated light chain (data not shown). Apparently complete restoration of surface IgG was seen in both pHeBo(A2) (lane b) and pHeBo(A1) (lane i) gene transfere nts, in parallel with the restoration of IgG secretion in these cell lines (Table 3). The pHPT vector control transfient (lane j) exhibited low but distinct surface immunoglobulin expression. However, this surface immunoglobulin expression was unusual in that (i) two heavy-chain bands at 72 and 61 kDa, as well as a faint band at 52 kDa, were precipitated, and (ii) very little labeled κ light chain was associated with the heavy chains. In contrast, normal surface IgG bands were precipitated from the pHPT(C) (lane k) and pHPT(B8) (lane l) transfere nts. HLA-C and -B transfere nts expressed different relative amounts of γm and γs: pHPT(C) expressed 4 times more γm than γs, whereas pHPT(B8) expressed 1.8 times more γs than γm, as measured by scanning densitometry. Predominance of

![Figure 1](https://example.com/figure1.png)

**Table 1**: Secretion of IgG1 and free κ by HLA class I gene and vector control transfere nts of the HLA-A**B**C**C** mutant .221

<table>
<thead>
<tr>
<th>Protein</th>
<th>pHeBo</th>
<th>pHeBo(A2)</th>
<th>pHeBo(A1)</th>
<th>pHPT32</th>
<th>pHPT(B8)</th>
<th>pHPT(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>&lt;1</td>
<td>779 (0.05)</td>
<td>810 (0)†</td>
<td>81 (0.06)</td>
<td>955 (0.02)</td>
<td>527 (0)†</td>
</tr>
<tr>
<td>κ</td>
<td>1014 (0.12)</td>
<td>1181 (0.23)</td>
<td>1402 (0.20)</td>
<td>355 (0.08)</td>
<td>2035 (0.06)</td>
<td>1080 (0.11)</td>
</tr>
<tr>
<td>κ/IgG ratio</td>
<td>&gt;10³</td>
<td>1.52</td>
<td>1.73</td>
<td>4.38</td>
<td>2.13</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Control transfere nts received only the pHeBo or only the pHPT32 vector. The identities of the LCL 721-derived genomic class I transgen es transferred with the vectors are indicated in parentheses.

*Mean (SD as fraction of mean) of triplicate well determinations. These determinations and those in Table 2 were made in the same experiment.

†Duplicate instead of triplicate determination.
surface γ vs. γm was also observed in protein A immunoprecipitation of a surface-labeled pHPT(B5) transferent (data not shown). It is clear from Fig. 1 that normal expression of surface IgG was partially or completely restored in mutant .221 along with restoration of IgG secretion (Table 3) by the transfer of a functional HLA-A, -B, or -C gene.

Flow Cytometry of Class I and IgG Expression on Mutant .221 and HLA-A, -B, or -C Gene Transferrers. To rule out aberrant class I expression in the cell lines tested (Tables 2 and 3; Fig. 1), we used flow cytometry to quantitate the binding of mAb PA 2.6 to the LCLs. The results were in uniform agreement with previously published measurements of class I expression on mutant, class I gene transferrers, and vector control-transferrers LCLs (20, 21). Thus, for example, mutants .184 and .221 exhibited 15% and 1.7%, respectively, of the surface class I expression found on LCL 721.

To confirm the absence of surface IgG in mutant .221 and its restoration by HLA-A, -B, -C gene transfer, we used both a goat anti-human IgG Fc-specific polyclonal antibody preparation and mAb 1410. Although quantitative measurements of surface IgG by flow cytometry differed substantially between the two reagents, both reagents confirmed the essential findings of Fig. 1: absence of surface IgG on mutant .221 and partially or fully restored surface IgG expression on HLA-A (A1, A2), -B (B5, B8), or -C gene transferrers (data not shown).

Intracellular Expression of γ and κ Chains of IgG in Mutant .221. The apparent absence of cell surface and secreted Ig γ chains in .221 made it important to determine whether γ chains were produced but retained within the cell. We approached this question in two ways: (i) ELISA analysis of γ and κ chains in cell lysates of mutant .221 (Table 4) and by immunoprecipitation of [35S]methionine-labeled proteins of mutant .221 (Fig. 2). The ELISA for IgG was modified from that we used to test all secreted IgG; plates were precoated with goat anti-human IgG (Fc-specific) instead of anti-F(ab')2, to capture both intact IgG and free γ chains. As shown in Table 4, mutant .221 expressed slightly more κ than LCL 721, but intracellular γ-chain expression was greatly reduced (2.2% of LCL 721), although significantly above that of the negative control LCL DAKIKI (IgA, λ).

One-dimensional reducing SDS/PAGE analysis of immunoprecipitates of metabolically labeled proteins showed that both the anti-γ and the anti-κ reagent precipitated associated γm (61 kDa), γ (52 kDa), and κ (26 kDa) chains from cell lysates (Fig. 2A). Free κ chains were also readily detected in immunoprecipitates of mutant .221 but, in contrast to LCL 721, neither the anti-γ nor anti-κ immunoprecipitates of .221 contained a prominent γm or γ band (Fig. 2B). While faint bands at 52 kDa were observed in .221, these may be similar to the 51/52-kDa doublet observable after immunoprecipitation of LCL 721 with an irrelevant antibody (Fig. 2A, lane m). Other pulse–chase experiments also failed to detect accumulation of a 52-kDa γ chain in mutant .221 at times 0, 0.5, or 4 hr after pulse labeling (data not shown).

Table 4. Mutant .221 has normal or increased amounts of cell-associated κ chain and a reduced amount of immunoactive γ chain relative to LCL 721

<table>
<thead>
<tr>
<th>Chain</th>
<th>LCL 721</th>
<th>.221</th>
<th>DAKIKI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td>1655 ± 314 (100%)</td>
<td>36.1 ± 25.9 (2.2%)</td>
<td>&lt;3 (&lt;0.2%)</td>
</tr>
<tr>
<td>κ</td>
<td>3458 ± 726 (100%)</td>
<td>4893 ± 1027 (141%)</td>
<td>&lt;7 (&lt;0.2%)</td>
</tr>
<tr>
<td>γ/κ ratio</td>
<td>2.08</td>
<td>135.5</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD of three separate experiments. SD of triplicate well determinations in all experiments was <20% of the mean.

†An Ig(A)-producing LCL.
The restorative effects of class I transgenes in .221 unmistakably implicate the expression of HLA-A, -B, and -C α chains in normal expression of the immunoglobulin γ chain in these LCLs. The involvement of class I α chains in normal immunoglobulin γ-chain production is emphasized by the partial restoration of γ production in .221 transfectants that express HLA-E and -F class I transgenes (ref. 21; S. Kovats and R.D., unpublished observations). This suggests the possibility that the normally very small amounts of intracellular non-A, -B, -C class I α chains that are present in .221 (20, 21) are idiosyncratically increased in the pHPT32 control transfectant, resulting in partial restoration of IgG production without a detectable increase in surface class I expression. The aberrant nature of this expression—e.g., the presence of a third, 72-kDa γ chain (γ3?)—remains puzzling.

We do not know of another B-cell line with deficient expression of HLA-A, -B, and -C that can be compared to mutant .221. Surface expression of HLA-A, -B, and -C antigens is deficient in the Daudi cell line because of a deficiency of β2-microglobulin (36). That Daudi cells have membrane IgM but do not secrete it (37) suggests that Daudi may export μ heavy chains by a class I-independent process or that the molecular defects in Daudi (β2 microglobulin deficiency) and in .221 (class I α-chain deficiency) may have different indirect effects on heavy-chain export.

Finally, a role for HLA class I molecules in production of IgG has not been previously described. Perhaps the severe agammaglobulinemia and lack of plasma-cell differentiation in the class I form of congenital "bare lymphocyte syndrome" (27, 28) are related to an HLA class I requirement for IgG production.

We thank Dr. Debra Hullett for making the initial observation that mutant .221 does not secrete IgG. We thank Lynn Devito and Melanie Beeska for technical assistance, Drs. Donna PaulNock and Pat Wathen for critical comments on the manuscript, and Sara Pitterle and Darlene Austin for secretarial assistance. This work was supported by National Institutes of Health Grants DK31774, AI26941, and AI15486 (R.D.) and by Institutional Research Grant IN-35-30-25 from the American Cancer Society. This is paper 3049 from the Laboratory of Genetics at the University of Wisconsin.