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Further Studies on Hybrid Cell-Surface Antigens Associated with Human Chromosome 11[1]

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Abstract—*A new human immunogenetic cell-surface activity associated with human chromosome 11 in the A_L human-Chinese hamster ovary cell hybrid is described. Like a_1, but not a_2, it is present on the human erythrocyte. By mutagenesis and selection, specific, stable, variants of the A_L hybrid have been prepared exhibiting various combinations of a_1, a_2, a_3, and lactic dehydrogenase A activities. The antigens of the A_L system can be demonstrated by the horseradish peroxidase system which offers a promising approach to scanning of tissue cells.*

INTRODUCTION

In previous papers, a series of hybrids between human and Chinese hamster ovary (CHO) cells containing the complete (or almost complete) CHO genome, together with particular human chromosomes, has been described. It was demonstrated that under the conditions of the single-cell plating procedure employed antibodies lethal to human cell-surface antigens in the presence of complement do not react significantly with those of the Chinese hamster cell (1). Therefore, it is possible to analyze the human cell-surface antigens due to the particular human chromosomes contained in each hybrid cell (2).

The first of such hybrids was shown to contain chromosome 11 as its only human chromosome, and its total complement of human cell-surface antigens which are lethal in the presence of specific antibody and comple-

[1]This paper is No. 6 in a series entitled "Genetics of Cell Surface Antigens." Earlier papers are listed in the references.

ment was designated as the A_L group. Other hybrids with different human antigens were demonstrated (3). The A_L set of antigens was selected for special study as a model system.

If antisera can be prepared which selectively react with some, but not all, of the individual cell-surface antigens, it becomes possible to resolve A_L into its components. It was possible to discriminate between the a_1 and a_2 antigens by means of the discovery that human red cells display the former but not the latter antigenic activity, whereas the other human cells, (such as the cultured human lymphoblast) display both. It was shown that an antiserum produced by injection of human red cells into a rabbit kills all cells containing a_1 but will not kill cells containing the a_2 antigen. An antiserum responding exclusively to a_2 resulted when an antiserum made in rabbits against the HeLa cell was adsorbed exhaustively with normal human red cells. By mutagenesis and selection, it proved possible to prepare all the four expected phenotypes as stable, cloned stocks derived from the parental A_L-J1 hybrid; $a_1^+a_2^+$, $a_1^+a_2^-$, $a_1^-a_2^+$, and $a_1^-a_2^-$ (4, 5). The present paper identifies a new antigenic activity on the A_L hybrid.

METHODS AND MATERIALS

Cells and Growth Medium. The cells employed in this study consist of CHO-Kl and its auxotrophic mutants (6, 7), the S3 HeLa cell, cultured human fibroblasts, the J1 clone of the A_L hybrid of human amniotic fibroblasts and Chinese hamster cells which contain human chromosome 11 as their sole human chromosome (4, 5), various mutants of this hybrid cell, and human red blood cells. All tissue culture cells were cultivated in F12 supplemented with 5–8% fetal calf serum (8).

Antisera Employed. Standard antisera were prepared as described earlier (3) by a series of two or three intramuscular injections into a set of rabbits of an antigen consisting of either 5×10^7 tissue culture cells or $2–50 \times 10^8$ human red blood cells, plus Freund's adjuvant, at 14-day intervals, the antiserum being collected 7–10 days after the final inoculation. A serum designated as "hyperimmune" was obtained by a further set of one or more intravenous injections of the same antigen. Appropriate increases in the amount of antigen were made when sheep immunization was employed (3). In some cases, globulin preparations from the respective antisera made in the horse against human lymphoblasts grown in culture were used. These were generously supplied by Drs. Kashawagi and McCalman of the Department of Surgery, University of Colorado Medical Center (9, 10).

Antiserum designated as anti-a_1 was prepared by standard immuniza-

tion of rabbits with human red blood cells (RBC). Antiserum designated anti-a_2 was prepared by exhaustive adsorption of anti-HeLa serum with human RBC (4). A 10% dilution of the serum in saline G was adsorbed with shaking for 2 hr at 37° with 5×10^9 human RBC/cm^3. The resulting antiserum no longer exhibited activity against the a_1 antigen by means of the standard single-cell survival measurements used in this study. The use of normal rabbit serum as a source of complement and its testing has been described earlier (3).

The great majority of the antisera against human cells employed here exhibited no appreciable lethal action against the parental CHO-Kl cell. Whenever a measureable toxicity for this cell was present, it was removed by the standard adsorption procedure using the CHO-Kl cells as adsorbent. In this way, it was ensured that the antigens studied arose from the presence in the hybrid of genetic loci on the human chromosomes present in the hybrid cell. Injection into a rabbit of RBC of blood types AB, A, or O, or M or N produced antisera identical in their behavior to the a_1 antigen.

Antiserum Titrations. Determination of a given cell's sensitivity to a given antiserum is carried out by means of single-cell survival curves using varying concentrations of the antiserum and a constant concentration of complement. In accordance with previous practice, a cell is designated as "sensitive" (+) to a given antiserum if 90% of the cells are killed by 0.10% or less of the given antiserum, and "resistant" (0) if no killing is observed under these conditions. Actually, 0 cells exhibited no killing even in concentrations several-fold greater than that needed to kill 90% of the sensitive cells. Details of these titrations have been described previously (3, 4). Anomalous survival curves, in which the decrease in cell survivors with increasing concentration of antiserum is halted or even reversed beyond a certain critical concentration of antiserum, were occasionally observed in certain experiments. These anomalies were infrequent, and their occurrence was associated with the use of antisera previously adsorbed with large numbers of cells, together with complement preparations containing appreciable toxicity, so that only a limited concentration of complement could be used in these experiments. These situations could be remedied by removal of high-molecular-weight components of such antisera by centrifugation at 100,000*g*.

Mutagenesis. Cells were treated with ethyl methane sulfonate, X irradiation, ICR-191E (an acridine mustard, kindly furnished by Dr. Creech), nitroso compounds, and other mutagens in accordance with the standard procedures previously described (7).

LDH-A Analysis. The presence of human lactic dehydrogenase A, an enzyme demonstrated to be on human chromosome 11, was determined by isozyme procedure as described previously (11).

Immunofluorescence and Immuno-Horseradish Peroxidase Procedures. The immunofluorescence procedure of Moller and Sorg (12, 13) gave good visual, but poorly photographable, results. Consequently, the horseradish peroxidase method was developed, with the generous assistance of Dr. Paul Nakane (14). In 1 ml of saline G, 3×10^4 lightly trypsinized cells were suspended (7) and deposited on a microscope slide by spinning in a cytocentrifuge for 12 min at 1000 rpm. The slides are placed in 1% formaldehyde diluted in PBS for 8 min at room temperature, then washed 3 times in saline G. The slides are then incubated for 30 min at room temperature with a 10% dilution in saline G of the appropriate antiserum on the one hand and an appropriate control serum on the other. The slides are again washed 3 times in saline G and then incubated for 30 min at room temperature in a standard solution (1%) of immunoglobulin prepared from goat antiserum to rabbit I_gG, as supplied by Colorado Serum Company, which had been coupled to horseradish peroxidase enzyme. After another set of washes, the slide is incubated in a 0.025% solution of 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M Tris-HCl buffer at pH 7.6 plus 0.001% H_2O_2 for 15–25 min at room temperature. Thereafter, the slides were washed 3 times in saline G, rinsed in distilled water, and mounted with Permount.

RESULTS

Production of a Clone Which is $a_1^- a_2^- a_3^+$. The availability of antisera which had been characterized as anti-a_1 and anti-a_2 (4), respectively, makes possible search for further cell-surface antigenic markers arising from loci on human chromosome 11. The rationale for this approach is as follows: (1) A population of A_L^+ hybrid cells is treated with a mutagenic agent under standard conditions. (2) After removal of the mutagen, the survivors which represent 2–20% of the original population are allowed to grow for several days in growth medium and then are placed in complement plus anti-a_1, anti-a_2, or both antisera. (3) Clones which develop under these conditions are those which have lost the capacity to be killed by the specific antibodies which kill the parental cell. Hence, they have presumably lost the ability to produce the crucial antigen tested for. Such clones are picked, grown into large cultures, whose resistance to killing by anti-a_1 and anti-a_2 and both together are confirmed. (4) Clones demonstrated to be impervious to the lethal action of both anti-a_1 and anti-a_2 sera are selected by this means. Such clones are by definition a_1^- a_2^-. They are then tested with the antisera against other human cells, such as the horse antihuman lymphoblast serum. If any of these clones are killed by the antihuman lymphoblast serum, they must possess one or more additional human surface antigens and the corresponding antibody must be

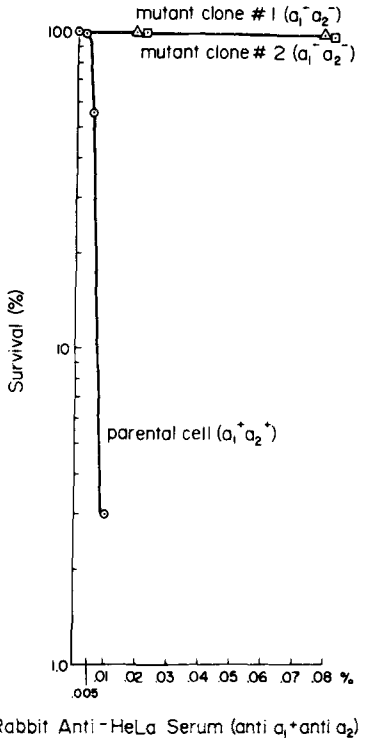

Fig. 1. Demonstration of the behavior of 2 mutant clones selected from the parental $a_1^+a_2^+$ population after mutagenesis and growth in the presence of anti-HeLa serum which contains lethal antibodies to both a_1 and a_2. The mutant clones 1 (A_L-J1-13) and 2 (A_L-J1-18) fail to be killed (0 reaction) by an antiserum which kills (+ reaction) the parental A_L hybrid cell which had been shown to be $a_1^+a_2^+$. Therefore, both mutant clones are designated $a_1^-a_2^-$. Standard complement was present in all titrations.

present in the antilymphoblast serum. Since this latter serum has no effect on the parental CHO-Kl cell, the new antigenic markers must also be controlled by loci on human chromosome 11.

Such experiments were carried out. The first new hybrid clone found by this procedure which is resistant to both anti-a_1 and anti-a_2, but which is killed by the antilymphoblast serum, was tentatively named a_3^+. Sample survival curves of this clone demonstrating its a_3^+ characteristic are presented in Figs. 1 and 2.

Demonstration that Human RBC Contained a_3. Injection of human RBC into rabbits by the standard immunization procedure produces an antiserum with strong anti-a_1, no anti-a_2, and little or no anti-a_3 activity. However, if injections are repeated so as to produce hyperimmune antiserum, quite high titers of a_1 and appreciably less high activities of a_3 are

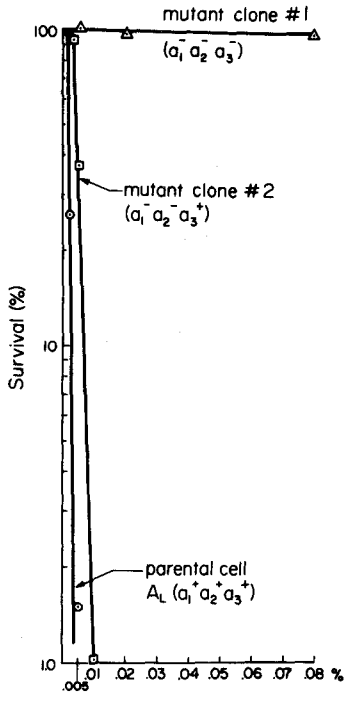

Fig. 2. Demonstration that the two similar mutant clones of Fig. 1 show divergent behavior when treated with antihuman lymphoblast serum. Clone 2 is killed almost as effectively as the parental cell (+ reaction) and so has been named $a_1^- a_2^- a_3^+$. Clone 1 is unaffected by eight times the amount of antiserum needed to kill 99% of the $a_1^- a_2^- a_3^+$ cells (0 reaction) and is therefore called $a_1^- a_2^- a_3^-$.

produced. Finally, if human RBC are injected into sheep, even the standard immunization procedure produces a high titer of activity against a_3. In confirmation of previous results (4), under no circumstances has anti-a_2 activity been found after injection of human RBC into animals. While individual animals exhibited the expected variations, the general pattern produced is illustrated in the data of Table 1. This differential action of sheep and rabbit response was confirmed in antisera obtained from two to five animals of each kind. It is possible that the human a_3 antigen chemically resembles some rabbit gene product sufficiently to make its immune response in that animal difficult to demonstrate.

The existence of the a_3 antigen on the A_L hybrid was initially demonstrated by means of the antiserum produced in the horse against a human lymphoblast cell culture employed as antigen. However, in subsequent horse immunizations with human lymphoblasts, the titer of the a_3

Table 1. Representative data demonstrating the effectiveness, for killing a_3^+ hybrids, of antisera against human RBC produced by standard immunization of sheep or hyperimmunization of rabbits, but not standard immunization of the rabbit[a]

Cell tested		Killing action displayed by survival curve response to following antisera to human RBC		
		Standard rabbit antiserum	Hyperimmunized rabbit antiserum	Standard sheep antiserum
A_L-J1 hybrid:	$a_1^+a_2^+a_3^+$	+	+	+
Mutants of the A_L hybrid obtained by mutagenesis and selection	$a_1^-a_2^-a_3^+$	0	+	+
	$a_1^-a_2^-a_3^-$	0	0	0
Parental CHO-Kl		0	0	0

[a]The + and 0 designations have their standard meanings as defined in the Methods section.

antibody obtained was found to be variable. In contrast, however, the response of sheep immunization to human RBC, in producing high titers of anti-a_1 and anti-a_3 activity, but no anti-a_2 activity, has been gratifyingly uniform.

The presence of a_1 and a_3 antigens on the surface of the human RBC was also demonstrated by the ability of human RBC to remove all killing activity from antisera against a_1 and a_3.

Preparation by Mutagenesis and Selection of A_L Hybrids with Different Combinations of Human Cell-Surface Antigens, a_1, a_2, and a_3. Antisera with different activities can be prepared by injection of tissue culture or biopsy cells from different human tissues, or of cultured hybrid cells into animals, and adsorption where appropriate with other cells to remove interfering activities. Table 2 illustrates some of the antisera which have been prepared by this means. Given a collection of antisera like those shown in Table 2, which are active against various combinations of a_1, a_2, and a_3, mutants displaying new phenotypes can be secured. Hybrid cell populations are subjected to mutagenesis and selection in the presence of antisera with particular activities so as to permit only clones with the desired phenotype to develop. The colonies which develop in the presence of an antiserum lethal to the parental cell have presumably lost certain parental antigens. Such colonies have been selected and their genetic stability demonstrated with respect to a_1, a_2, and a_3 antigens. Both single-step and repeated-step mutagenesis experiments have been carried out. Thus, while some phenotypes like $a_1^-a_2^-a_3^+$ have been produced by a single mutagenesis treatment, better yields are obtained in a two-step procedure, as when $a_1^+a_2^-a_3^+$ or $a_1^-a_2^+a_3^+$ are mutagenized further. With

Table 2. Demonstration of preparation of representative antisera with specific activities toward a_1, a_2, and a_3 antigens[a]

Cell used as antigen	Animal host	Standard (S) or hyper (H) immunization	Cell used for adsorption	Presence in the final preparation of specific antibody against		
				a_1	a_2	a_3
Human RBC	Rabbit	S	None	+	0	0
	Rabbit	H	None	+	0	+
	Sheep	S	None	+	0	+
S3-HeLa	Rabbit	S	None	+	+	0
	Rabbit	S	Human RBC	0	+	0
Human lymphoblast	Horse	H	None	+	+	+
	Horse	H	Human RBC	0	+	0
Human fibroblast culture	Rabbit	S	None	+	+	0
$a_1^-a_2^+a_3^-$ hybrid	Rabbit	S	CHO-Kl	0	+	0
$a_1^+a_2^-a_3^+$ hybrid	Rabbit	H	CHO-Kl	+	0	0

[a]The "+" means the given antiserum at a concentration of 0.1% or less in the presence of standard complement kills 90% or more of a test cell carrying the given antigen. The 0 means no or virtually no killing is observed under these conditions.

three independent markers, eight different phenotypes would be expected. Table 3 demonstrates that six of eight possible a_1, a_2, and a_3 combinations have already been prepared. All of the resulting clones have displayed stable antigenic properties throughout many months of cultivation in the absence of the selective media used in their original isolation. Not all of the clones listed in Table 3 are obtained with equal frequencies, a fact which may be related to the nature of the genetic change underlying their formations, since some of these are deletions, while others may be individual mutations. Where more than one genetic marker has been lost in a single mutagenesis treatment, a deletion may have occurred, although other genetic mechanisms which may be operative in certain situations are also under study. Karyotypic study of representative mutants is described in the following paper of this series (15). Quantitative aspects of these mutagenic actions which should illuminate the details of the processes that occur under the influence of different mutagens are also under study.

The failure so far to isolate clones with the phenotype $a_1^+a_2^+a_3^-$ and $a_1^+a_2^-a_3^-$ is not necessarily significant because optimal antisera effective for these particular isolation operations have not been available. Since se-

Table 3. Examples of the varieties of stable variant clones obtained by mutagenesis of $a_1^+a_2^+a_3^+$ hybrids in single or multiple steps[a]

Clone no.	Mutagenic agent employed	Antibody activity used in original clone isolation	Presence of specific antigen			Presence of human LDH-A
			a_1	a_2	a_3	
A_L-J1	None	None	+	+	+	+
A_L-J1-3	EMS	a_1	−	+	+	+
A_L-J1-22	EMS	a_1	−	+	+	−
A_L-J1-11	ICR	a_2	+	−	+	+
A_L-J1-18	EMS	a_1	−	−	+	+
A_L-J1-21	X-ray	$a_1 + a_3$	−	+	−	+
A_L-J1-7	X-ray	$a_1 + a_3$	−	+	−	−
A_L-J1-13	ICR	$a_1 + a_2$	−	−	−	+
A_L-J1-5	EMS	$a_1 + a_2$	−	−	−	−

[a]In each case, the parental hybrid was treated with a mutagen under the conditions previously described, and the resulting cell population was plated in the presence of various antisera active against different combinations of the three antigens. Surviving clones were picked, established into new stocks, and also tested by isozyme analysis for their possession of human lactic dehydrogenase A activity. A representative of each mutational class isolated is shown.

lective techniques for obtaining clones exhibiting LDH-A^+ or LDH-A^- behavior are not yet available, the LDH-A status of the clones selected by immunologic means shown in Table 3 is determined largely by chance. However, loss of LDH-A was observed more frequently to parallel loss of a_1 and a_3 than a_2, an observation explained by the data of the following paper in this series.

Antisera can also be produced by injection into experimental animals of the variant hybrid cells listed in Table 3. When such hybrids are injected into rabbits or sheep, antibodies to Chinese hamster cell components will also be produced which are removed by adsorption with the parental CHO cell which was used in the original formation of the hybrid. Usually the antisera so prepared display the activities expected on the basis of the antigens assigned to the injected cell as shown in Table 2 for the $a_1^-a_2^+a_3^-$ cell. However, occasionally a hoped-for activity fails to materialize, as indicated in the last line of Table 2, in which activity against the a_3^+ antigen was not found. This may not be too surprising in view of the rabbit's relatively poor response to the a_3 antigen.

Chromosomal Specificity of These Antigenic Effects. The elucidation of chromosomal specificity of cell-surface antigenic structures involves the question: Can expression of genetic determinants for surface antigens on one chromosome be affected by the presence of other chromosomes? The simultaneous presence of other chromosomes has not yet revealed any regulatory activity on A_L expression. Figure 3 demonstrates that a

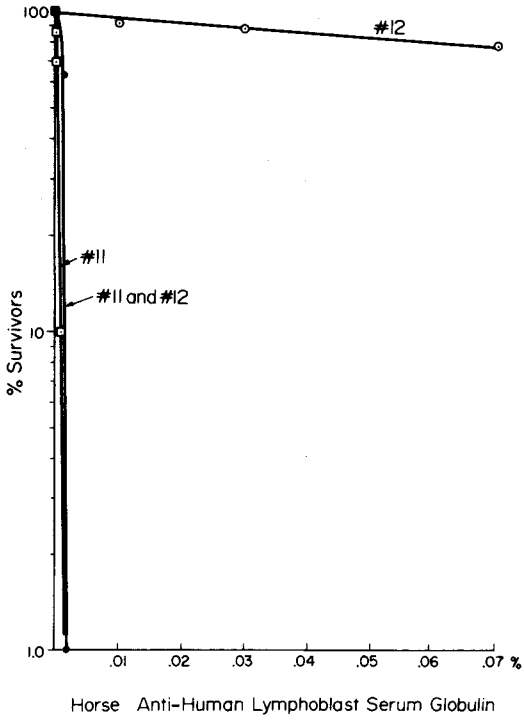

Fig. 3. Demonstration that horse anti-human lymphoblast serum, which contains antibody activity against a_1, a_2, and a_3 antigens of the A_L system, fails to kill the hybrid containing human chromosome 12 as its only human chromosome and kills a hybrid containing both human chromosomes 11 and 12 with the same effectiveness as the A_L hybrid which contains 11 only.

hybrid containing only human chromosome 12 is not affected by an antiserum containing antibodies against all the antigens of the A_L hybrid. Also, a hybrid containing human chromosome 12 in addition to human chromosome 11 is still sensitive to the antiserum against A_L. The chromosome constitutions of these hybrids have been described elsewhere (5). As larger numbers of hybrids are accumulated, the possibility of exceptions to this simple specificity, which would indicate interaction between chromosomal constituents of a cell, is being sought.

Direct Visualization of Cell-Surface Antigens. The use of single-cell survival curves detects only complement-binding cell-killing antibodies. Moreover, while it can be applied to the detection of specific antibodies on the cells of normal and pathologic tissues, its use in this connection is inconvenient. Therefore direct visualization techniques were explored. While immunofluorescence appeared to give good results, the horseradish

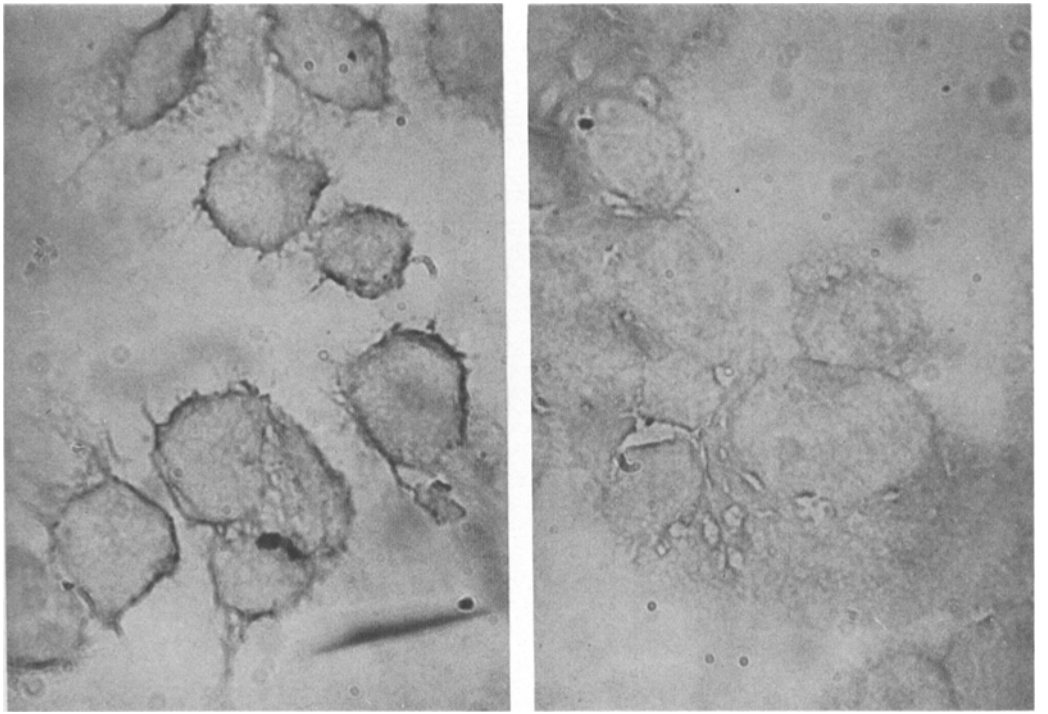

Fig. 4. Typical results obtained by the immuno-horseradish peroxidase reaction applied to the A_L cell-surface antigen system. (A) Treatment of an $a_1^+a_2^+a_3^+$ cell with an anti-a_1 serum (rabbit, antiserum against human RBC); (B) control situation in which the same cell has been treated with normal rabbit serum.

peroxidase procedure has so far proved more definitive. A typical result is presented in Fig. 4, which demonstrates the feasibility of this approach for such studies.

DISCUSSION

The experiments presented here reveal the presence of a different kind of cell-surface antigenic activity which we tentatively designate a_3. The nature of this new activity, characterized by the ability of a particular antiserum to kill hybrid A_L cells which have been mutagenized so as to lose their sensitivity to antisera against a_1 and a_2 under the standard conditions of our test, is still not completely defined. This behavior might be due to: a_3 as a new immunogenetic locus, distinct from a_1 and a_2; a_3 as an allelic form of a_1; a_3 as identical to a_1 but existing in a much lower concentration on cells designated as $a_1^-a_3^+$; or a_3 as involving unresolved genetic complexities, possibly including regulatory or other kinds of genetic and physiologic interactions.

The possibility of genetic complexity in these loci is appreciable since cell membrane structures like glycophorin and blood group substances are often complex molecules which require a variety of gene actions for achievement of their completed form and which may display more than a single antigenic activity. Further analysis to resolve the nature of a_1, a_2, and a_3, chemical constitution, and relationships is under way. In the meantime, as a working hypothesis, we adopt the first of the proposed explanations on the basis of its being the simplest scheme consistent with the known facts and because a mutation has been found in which the activities of a_1 and LDH-A but not a_3 have been lost (Table 3). Thus it would appear probable that this is a deletion mutation. Morever, occasional antisera have killed $a_1^-a_2^-a_3^+$ at least as well or even more effectively than $a_1^+a_2^-a_3^+$, a situation not easily reconciled with the a_3^+ character as simply a reduced amount of a_1. Preliminary results of complementation analysis to be described elsewhere support this interpretation. However, a final interpretation will not be attempted until further studies on chemical analysis, mutation frequencies, and complementation analysis have been completed.

Other human cell-surface antigens due to loci on human chromosomes 7 (16), 6 (17), 15 (18), and X (19) have been described by other investigators. In preliminary communications, Bodmer and his coworkers (20), have described antigenic activity associated with chromosome 11, whose relationship to the antigens of this study is considered in the next paper of this series.

In the accompanying paper in this series (15), regional mapping of the A_L loci is described. The approach described in this series of papers ap-

pears to be generally applicable to immunogenetic surface markers arising from genes on many human chromosomes. The only limits to the use of these methodologies which can be seen at the present time are the number of hybrids available containing single human chromosomes and the degree to which immunological similarity may hinder separation of certain human gene products from their Chinese hamster counterparts. As described elsewhere, we have already prepared human–CHO hybrids containing the single human chromosomes (or simple combinations of these) 8, 11, 12, 14, and 21, and one hybrid has been described containing a human chromosome for which it has not yet been possible to decide whether it is number 4 or 5 (21). Since more than 25 different CHO auxotrophs have already been prepared (7), it would appear that a reasonably large number of such hybrids containing different single human chromosomes may be possible. By utilizing different animals for immunization, it should be possible to maximize differences between the immune responses to similar but not identical antigens of man and other animals.

The general approach utilized in these studies has the advantage of permitting simultaneous genetic analysis for each cell-surface structure identified. If a single human chromosomal hybrid is utilized, the chromosomal location of the genetic locus is specified and regional mapping can be carried out. Complementation analysis which will be presented elsewhere has been performed by the methods developed for use with somatic cells (22) and demonstrates the genetic complexity governing expression of these loci. Biochemical identification of these antigens can also be performed, and the specific antisera prepared in the course of these studies, as described here, can be an important tool in simplifying biochemical identification of each antigen.

The procedures here employed utilize antisera prepared both from antigens consisting of particular human cells and from hybrids containing specific human chromosomes. While in the former case it might be argued that the antisera so produced will contain antibodies due to many human surface antigens arising from loci on various chromosomes, this situation is in fact an advantage. If a given antibody activity is specifically removed by adsorption with hybrids containing human chromosome 11 and no other hybrids, it appears safe to relate such activity to gene products involving loci on human chromosome 11. If, in the future, complications or contradictions should arise by use of this procedure, their elucidation may well furnish important insights into genetic regulatory processes involving differentiation. The plan pursued in these studies has already demonstrated that the a_1 and a_2 antigens, both associated with human chromosome 11, possess different tissue cell distributions in the body. Hopefully more relationships of this kind will be forthcoming.

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LITERATURE CITED

1. Oda, M., and Puck, T. T. (1961). *J. Exp. Med.* **113**:599–610.
2. Puck, T. T., Wuthier, P., Jones, C., and Kao, F. T. (1971). *Proc. Natl. Acad. Sci. USA* **68**:3102–3106.
3. Wuthier, P., Jones, C., and Puck, T. T. (1973). *J. Exp. Med.* **138**:229–244.
4. Jones, C. Wuthier, P., and Puck, T. T. (1975). *Somat. Cell Genet.* **1**:235–246.
5. Kao, F. T., Jones, C., and Puck, T. T. (1976). *Proc. Natl. Acad. Sci. USA* **73**:193–197.
6. Kao, F. T., and Puck, T. T. (1969). *J. Cell Physiol.* **74**:245–258.
7. Kao, F. T., and Puck, T. T. (1974). In *Methods in Cell Biology, Vol. VIII*, (ed.), Prescott, D. M. (Academic Press, New York) pp. 23–39.
8. Ham, R. G. (1965). *Proc Natl. Acad. Sci. USA* **53**:288–293.
9. Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zühlke, V., and Starzl, T. E., (1967). *Surg. Gynecol. Obstet.* **124**:1–24.
10. Groth, C. G., Kashiwagi, N., Moore, G. E., Husberg, B., Bjorken, C., Putnam, C. W., and Starzl, T. E. (1972). *Behring Inst. Mitt.* **51**:86–89.
11. Jones, C., Wuthier, P., Kao, F. T., and Puck, T. T. (1972). *J. Cell Physiol.* **80**:291–297.
12. Moller, G. (1961). *J. Exp. Med.* **114**:415–432.
13. Sorg, C. (1974). *Eur. J. Immunol.* **4**:832–834.
14. Nakane, P. K., and Pierce, G. B. (1966). *J. Histochem. Cytochem.* **14**:929–931.
15. Kao, F. T., Jones, C., and Puck, T. T. (1976). *Somat. Cell Genet.* **3**:421–429.
16. Aden, D. P., and Knowles, B. B. (1976). *Immunogenetics* **3**:209–221.
17. Van Someren, H., Westerveld, A., Hagemeyer, A., Mees, J. R., Meera Khan, P., and Zaalberg, O. B. (1974). *Proc. Natl. Acad. Sci. USA* **71**:962–965.
18. Goodfellow, P. N., Jones, E. A., Van Heyningen, V., Solomon, E., Bobrow, M., Miggiano, V., and Bodmer, W. F. (1975). *Nature* **254**:267–269.
19. Buck, D. W., and Bodmer, W. F. (1976). Third International Workshop on Human Gene Mapping. Birth Defects: Original Article Series Vol. 12, No. 7, (The National Foundation, New York) pp. 376–377.
20. Buck, D. W., and Bodmer, W. F. (1974). Second International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. 11, No. 3, (The National Foundation, New York) pp. 87–89.
21. Kao, F. T., and Puck, T. T. (1972). *Proc. Natl. Acad. Sci. USA* **69**:3273–3277.
22. Kao, F. T., Johnson, R. T., and Puck, T. T. (1969). *Science* **164**:312–314.