Antigenicity of Canine Tissue Cultures[1] (34241)

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Ten years ago, Coriell (1) suggested that continuous cell culture lines acquire common antigens that frequently cross species lines. Later Brand and Syverton (2) using the hemagglutinating activity of antitissue culture serum found that many continuous lines purportedly derived from man, monkey, rabbit, pig, calf, hamster, or duck were usually human or mouse cells. All continuous cell lines developed in laboratories in which HeLa or mouse L cell lines were also maintained were then suspected of being the result of contamination with fast growing cells until conclusively proven otherwise.

Some preliminary immunologic investigations of a continuous line developed in our laboratory from beagle dog kidney showed it to be similar to HeLa cells, yet to retain certain characteristics of dog cells not found in the HeLa line. It occurred to us that tissue cultures from many different species might develop common antigens as they were passaged becoming more immunologically similar as they developed into continuous lines.

The purpose of this study was to determine if such antigens do develop with growth, if species-specific antigens are retained, and at what point these changes occur. Since species-specific antigens may be determined by the direct agglutination of homologous erythrocytes (2), and by the indirect mixed agglutination (3), these and an indirect fluorescent antibody technique were used to compare dog kidney cell cultures in various stages of growth with human amnion and HeLa cell cultures.

TABLE I. Tissues and Cultures Used.

Symbol	Origin	
DK	Beagle dog kidney	Uncultured
PDK	Beagle dog kidney	Primary
PDK_3	Beagle dog kidney	Third passage
MDCK	Dog kidney	Continuous line[a]
DK-L	Beagle dog kidney	Continuous line[b]
PHA	Human amnion	Primary
HeLa	Human uterus	Continuous line[c]

[a] Obtained from Industrial Biological Laboratories. Chromosomes similar to dog cells.

[b] Developed by F. F. Pindak in the Microbiology Department, Lovelace Foundation. Chromosomes similar to HeLa cells.

[c] Obtained from Dr. L. O. McClaren, University of New Mexico Medical School.

Materials and Methods. Cell cultures. The cell cultures used are listed in Table I. Medium 199[3] with 10% inactivated calf serum, 200 units of penicillin, 100 μg of streptomycin, 100 μg of neomycin and 6 μg of amphotericin B/ml was the growth medium for primary dog kidney (PDK), dog kidney third passage (PDK_3), dog kidney-continous line (DK-L) and HeLa cultures. For primary human amnion (PHA) cells 20% calf serum was used. The continuous line monkey (MDCK) cell cultures were grown in Eagles' minimum essential medium (MEM) made in Hanks' balanced salt solution with 10% inactivated fetal calf serum. Maintenance medium consisted of MEM with 1% calf serum. Dog kidneys were obtained from newborn to 1-month-old beagle pups and were prepared for culture by the method of Younger (4). Human placentas were processed as suggested by Parker (5).

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Preparation of antigens and antisera. Tissue culture antigens were obtained by harvesting with Versene (ethylenediaminetetraacetic acid) with the exception of PHA cultures which were removed with trypsin. Suspensions of these cells and of uncultured dog kidney were prepared and injected into rabbits by methods described by Milgrom *et al.* (3). Dog and human kidney powder for absorption of antisera were also prepared by their methods.

Direct hemagglutinations. The method used was that described by Holmgren and Payne (6). The rabbit antisera against the specific tissues were reacted with beagle dog and human O erythrocytes. Sheep erythrocytes were also used to determine Forssman antibodies. When a titer of more than 1:16 was observed, that serum was absorbed with guinea pig kidney.[4]

Mixed agglutination in tissue cultures. The procedure followed was essentially that of Milgrom *et al.* (3). Tissue cultures under investigation were grown in tubes. The cell monolayer was overlaid with dilutions of antiserum in medium 199. The antisera were those made by immunizing rabbits against the six cell cultures and uncultured dog kidney. The antiserum combined with specific antigens present in the tissue cultures. In order to demonstrate the reaction, sheep erythrocytes sensitized with rabbit antisheep erythrocyte serum and goat antirabbit serum were added after the cultures had been washed free of any unattached antitissue culture serum. Interaction of the goat antirabbit serum[5] with the rabbit serum combined with the cell culture caused the sheep cells to be absorbed on the surface and this was observed microscopically and compared to controls. Titrations were made with unabsorbed serum and with serum absorbed with dog (DK_P) and human kidney powder (HK_P).

Indirect fluorescent antibody techniques. The method of Lennette *et al.* (7) was used. Cell suspensions were dried on slides, fixed in acetone and stored at −60° until used. The cells on the slide were covered with rabbit antiserum against a specific tissue. After incubation and washing with buffer, they were covered with fluorescein tagged goat antirabbit-globulin.[6] This reacts with any of the original rabbit antiserum which combined with antigens in the tissue culture cells. The slides were then examined microscopically for fluorescence under ultraviolet light. They were scored blind and the intensity of the fluorescence was expressed at 1+ through 4+ as compared to positive and negative controls. Controls with normal rabbit serum and others with conjugate alone were included with each set of tests.

TABLE II. Human and Dog Hemagglutinin Titers of Rabbit Antisera to Human and Dog Tissue.[a]

	Serum absorbed with guinea pig kidney		Unabsorbed serum
	Red blood cells		
Antiserum to	Human	Dog	Sheep
DK	0	256	32
PDK	128	256	128
PDK_3	256	256	256
MDCK	256	256	128
DK-L	256[b]	0[b]	4
PHA	1024[b]	0[b]	16
HeLa	512	<64[c]	16

[a] Values are reciprocals of the titers.

[b] Titers are for unabsorbed serum since hemagglutinin titers for sheep cells were 1:16 or less.

[c] Dilutions less than 1:64 of absorbed sera hemolyzed dog red blood cells.

Results and Discussion. Species-specific antigens. Table II shows that antiserum against uncultured dog kidney agglutinated dog but not human red blood cells. Antiserum to primary human amnion agglutinated human but not dog erythrocytes. The reactions with anti-DK-L and anti-HeLa serum indicate that they are both of human origin, although the original DK-L cells were derived from a culture of dog kidney tissue. However, the titers of the antisera to PDK, PDK_3, and MDCK are approximately equal using either human or dog erythrocytes. This would suggest (a) that these three cultures

[4] Hyland Laboratories, Los Angeles, California.

[5] Colorado Serum Company, Denver, Colorado.

[6] Obtained from Arnel Products, New York, New York.

are a mixture of cells of human and canine origin, or (b) that with passage the cultured cells acquire antigens which are similar to those in human red blood cells, or (c) that human and dog erythrocytes share some common antigen (8). Contamination of primary and tertiary cell cultures in such a short period and under the carefully protected conditions maintained in this experiment seems most unlikely. These cells did not resemble HeLa morphologically nor did any of the cultures used in this study develop into a continuous line. If contamination with HeLa cells had occurred, it is presumed that these rapidly growing cells would have replaced the slower growing dog cells. Although all the antisera to the dog tissue and cell cultures showed titers of 1:32 to 1:256 to sheep cells (Forssman antibody), these were lowered to less than 1:8 by absorption with guinea pig kidney. The absorbed serum was used to obtain the values discussed above (Table II) and preabsorption titers to human and dog erythrocytes were not lowered more than one dilution.

The presence of antigens that are apparently species-specific was also demonstrated by the mixed hemagglutination technique (Table III). In the first column the titers of uncultured dog kidney (DK) antiserum are shown against six tissue cultures. Unexplainably only a trace titer could be found with MDCK but activity for PDK, PDK_3, and DK-L was found and an appreciable lowering of titer was observed after absorption with dog kidney powder. No activity was shown with the two human cultures. It is of some interest that the DK-L culture which appears to be of human origin by the direct hemagglutination test here shows some evidence of a previous canine relationship. The titer is reduced to zero by absorption with dog kidney powder.

The decrease of species-specific antigen with increasing culture passages is shown by fluorescent antibody reactions (FA) in Table IV. The anti-DK serum reacts most strongly with PDK and shows decreasing activity with the proven continuous line of dog kidney (MDCK), the questionable DK-L line and none with the human cells. Again a sug-

TABLE III. Mixed Hemagglutinin Titers (log 10) to Dog and Human Tissues.[a]

Antiserum:	DK		PDK		PDK_3		MDCK		DK-L		PHA		HeLa	
Absorbed with:	O	DK_p	O	DK_p	O	DK_p	O	DK_p	HK_p	O	DK_p	HK_p	O	HK_p	O	HK_p
Tissues																
1 PDK	4	tr	5	4	5	4	5	4	4.5	tr	tr	tr	0		3.5	3
2 PDK_3	4.5	3	5	4	5	4	5	4.5	5	4.5	3	3	0		4.5	4
3 MDCK	tr	tr	4	tr	4	3.5	5	4	4	tr	tr	tr	tr	tr	tr	
4 DK-L	3.5	0	4	3	4	4	4	3	4	5	5	5	5	4	5	5
5 PHA	0		0		0		tr	tr	tr	3	tr	tr	4	3.5	4	4
6 HeLa	0		3	3	3.5	3	4	3	3	5	4.5	3	4	tr	5	5

[a] tr = trace reaction in 10^{-3} dilution; lower dilutions gave negative hemagglutination; DK_p = dog kidney powder; HK_p = human kidney powder.

TABLE IV. Fluorescent Antibody Reaction of Dog and Human Antisera to Dog and Human Tissue.[a]

Tissue cultures	Anti-serum dilutions	Antisera						
		DK	PDK	PDK_3	MDCK	DK-L	PHA	HeLa
PDK	10^{-1}	++++	++++	++++	++++	++	++	+++
	10^{-2}	++	+++	++++	++	±	±	+
	10^{-3}	0	+	+	±	0	0	0
PDK_3	10^{-1}	++++	++++	++++	++++	±	0	+++
	10^{-2}	+	+++	++++	++	0	0	±
	10^{-3}	0	±	++	0	0	0	0
MDCK	10^{-1}	++	++++	+++	++++	0	±	+
	10^{-2}	0	+	+	+	0	0	0
	10^{-3}	0	0	0	0	0	0	0
DK-L	10^{-1}	+	++	+	±	++++	+	++++
	10^{-2}	0	0	0	0	+	0	+
	10^{-3}	0	0	0	0	0	0	0
PHA	10^{-1}	0	+	0	++	+	++++	+++
	10^{-2}	0	0	0	0	0	+++	+
	10^{-3}	0	0	0	0	0	+	±
HeLa	10^{-1}	0	+	++	+	++	++	++++
	10^{-2}	0	0	0	0	0	±	+++
	10^{-3}	0	0	0	0	0	0	±

[a] Key: 0 = no fluorescence; ++++ = brilliant fluorescence.

gestion of a relation of DK-L cells to the canine species was observed with this reaction. However, the activity of HeLa antiserum to DK-L cells was as pronounced as that of the homologous antiserum. The determination of the species of origin by fluorescein tagged antibodies has been recommended by others (9, 10). Immunization of rabbits with uncultured dog tissue appears to produce highly species-specific antiserum as shown by the FA reaction and is possibly the most convenient way of confirming the canine origin of tissue cultures.

Acquired antigens. Human specific antigens. Primary dog tissue appears to have acquired human hemagglutinins during growth *in vitro* and before being passaged (Table II). Antisera to tertiary dog kidney cells, and the continuous lines derived from dog kidney (MDCK and DK-L), have the same or slightly higher human hemagglutinin titers and they are only slightly lower than those shown by antisera to cells of known human origin (PHA or HeLa).

Kornstadt and Rose (8) and Kite *et al.* (11) observed increases in titer to the red blood cells of several different species when HeLa and mouse L cells were used to immunize rabbits. They suggested that this may be due to cross reactions between antigens already present in the erythrocytes. Although we could not detect human-specific antigens on uncultured dog kidney (antiserum to this antigen did not react with primary human amnion by mixed hemagglutination or by fluorescent antibody techniques and did not agglutinate human red cells), it is possible that traces of human-specific antigen not detectable by these methods were present and that these antigens increased in quantity as the dog kidney was subcultured.

Forssman antigen. Table II shows an increase in antibody titer to sheep cells when dog kidney tissue is cultured. All of this activity was absorbed with guinea pig kidney. Forssman antigen is known to be present in many animals including the dog. To our knowledge, an increase in cultures has not been reported.

Other common antigens. The mixed he-

magglutinin titers (Table III) suggest that the cultured dog kidney cells share common antigens with HeLa cells. In the sixth line of Table III where HeLa antigen is tested against all antitissue sera, there is an increase in titer from zero with uncultured dog kidney to 10^{-3} with PDK, $10^{-3.5}$ with PDK_3, 10^{-4} with MDCK and 10^{-5} with DK-L which is the same as the homologous serum. In the other hemagglutinin titers as well as those shown in Table IV for FA, there is a sharing of antigens between tissue cultures which, in most cases, becomes greater from uncultured tissue-to primary-to continuous lines. Since all our tissues were grown in media with calf serum, the increase in antigens with growth might be suspected to be those derived from this source. Acquisition of serum antigens by cell cultures has been observed (11–14). However, in our study in several instances there was no reaction between tissue and heterologous antiserum. Furthermore, in two cases, anti-PDK against MDCK cells and anti-PHA against HeLa cells, absorption with the homologous kidney antigen reduced the reaction to a trace. This could not have occurred if calf serum antigen were a significant contributing factor to the original titer.

Antigens common to continuous cell lines have been reported before (1, 6, 11, 15). Most of these were cultures derived from human or primates. A "human stable line" (HSL) antigen (16) and the G tumor antigen of HeLa and other cells (17) have been characterized. It is possible that all or most continuous lines develop a common antigen related to tumorogenicity (6, 16).

Infection with virus or mycoplasma may result in the appearance of a common antigen (18, 19). No mycoplasma were isolated from the cultures studied, although using the same methods, mycoplasmas were isolated from a cell line not used in this study. It seems unlikely that all of the tissues studied would be infected with the same agent and this be responsible for the common antigen. A similar antigenic change might be brought about by different agents however.

Gradual increase of antigen in primary human amnion tissue culture was reported by Holmgren and Payne (6). This antigen was not detectable until after several days growth. It was measured with hemagglutinin free anti-HeLa serum by the FA technique. This is similar to our own observations in growing dog kidney tissue cultures.

Apparently antigens may be acquired and shared by growing cultures of cells from different species. Because of this the species of origin of a continuous line may be obscured such that it appears to be a line derived from another species. Is this, in fact, a contamination or is it an acquisition of antigens which are common to most continuous cell lines and which are in evidence in lesser amount soon after the cells begin their growth *in vitro*? In the case of our DK-L cells, the question arises whether changes occurred in the early passages which were more favorable to growth in the new environment until finally by selection the population resembles HeLa cells which were also derived in the same fashion but from a different source. If the DK-L line is indeed the result of contamination with HeLa cells then the remaining canine characteristics (Table III and IV) might be attributed to contact of the HeLa cell with the original dog kidney cells from which new canine-species antigen was taken up in small quantities.

Summary. Direct agglutination of erythrocytes, mixed hemagglutination, and FA techniques were used to study antigens in four cultures of canine and two of human origin. All cultures maintained species-specific antigens. Dog kidney cell cultures apparently acquired heterologous-species antigens during primary growth. These were antigens shared with human and sheep erythrocytes. Nonspecies-specific common antigens were found in the primary and tertiary dog kidney cells and the three continuous lines. The indirect FA test, using uncultured dog kidney tissue antiserum, correlated well with the indirect hemagglutination method.

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