

## *Brief Definitive Report*

### ALLOGENEIC AND XENOGENEIC RESPONSE IN MIXED LEUKOCYTE CULTURES\*

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Reactivity in allogeneic mixed leukocyte culture (MLC)<sup>1</sup> tests is usually regarded as a reflection of differences for the major histocompatibility system (1, 2), although exceptions to this general rule have been reported (3). The MLC response can be interpreted as the recognition (sensitization) phase of the *in vivo* homograft or graft-*versus*-host reaction (4, 5). Recently Wilson and Nowell (6) have shown that xenogeneic MLC stimulation is weak compared to that in allogeneic mixtures, as is the case in xenogeneic and allogeneic graft-*versus*-host responses (7). The lesser graft-*versus*-host responses in xenogeneic combinations could be explained in a number of ways. One possibility is that xenogeneic differences are less well recognized, an interpretation consistent with the above MLC findings.

However, since other possible explanations exist, it seemed important to confirm this lower MLC stimulation in xenogeneic combinations in a larger number of species in combinatorial fashion. We have tested the response of cells of three different species (human, mouse, and dog) to stimulation by these same three species plus two more (rabbit and rat). With the first three species we did combinatorial experiments; in all experiments we tested to show that cells included in the experiment could respond or stimulate in some allogeneic or xenogeneic combination.

MLC tests were done using a micromethod recently described for human cultures (8, 9). Human, mouse, and dog responding cells were used at concentrations which give optimal response for allogeneic mixtures. All cultures were done in RPMI 1640 supplemented with plasma, penicillin, and streptomycin. Human plasma was used with human and mouse responding cells; dog plasma was used with dog cells. Stimulating cells were treated with mitomycin C and tested at two different concentrations, again those which gave maximal allogeneic stimulation and those which on preliminary testing appeared to give good xenogeneic stimulation. Cultures were labeled with tritiated thymidine for 16 hr several days after the initiation of culture, again at a time when cells in the allogeneic mixtures were presumably in a phase of exponential growth as previously discussed (8).

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<sup>1</sup> *Abbreviation used in this paper:* MLC, mixed leukocyte culture.

As reported by Wilson and Nowell (6), we have observed cases in which xenogeneic combinations showed very little or no response; however, in each of these experiments other xenogeneic combinations did show responses sometimes equal to or even exceeding the allogeneic response. The data presented in Table I are from two experiments in each of which cells of two humans, two dogs, and two mice were tested in all combinations. To simplify the presentation, only one responding cell for each species is included.

In experiment I, the human cells respond more vigorously in the allogeneic mixture than in the xenogeneic ones; however, the xenogeneic combinations do show extensive proliferation. Likewise there is significant stimulation by

TABLE I  
*Allogeneic and Xenogeneic MLC*

Stimulating cells		Responding cells		
Experiment I	Human 1	Dog 1		Mouse 1
Human 1	(2611 ± 484)*	13,953 ±	6722	6584 ± 4054
Human 2	37,703 ± 1827	33,019 ±	13,074	61,186 ± 7264
Dog 1	14,404 ± 2627	(396 ±	106)	26,247 ± 3118
Dog 2	25,804 ± 1406	14,701 ±	4762	15,006 ± 4024
Mouse 1	9831 ± 1646	2462 ±	937	(6298 ± 2886)
Mouse 2	16,674 ± 2307	874 ±	272	83,300 ± 2427
Experiment II	Human 3	Dog 3		Mouse 3
Human 3	(641 ± 163)	35,381 ±	9520	38,657 ± 7750
Human 4	27,226 ± 1764	9265 ±	1571	34,987 ± 4111
Dog 3	851 ± 231	(329 ±	236)	45,014 ± 4993
Dog 4	1863 ± 277	14,696 ±	3874	40,259 ± 7017
Mouse 3	1202 ± 332	1247 ±	349	(1992 ± 940)
Mouse 4	1118 ± 269	2588 ±	1151	31,471 ± 1528

\* Counts per minute ± standard deviation. Isogenic control values are given in parentheses.

both the allogeneic and the xenogeneic stimulating cells with the mouse responding cells. The mouse-mouse and the one mouse-human mixture stimulate to about the same extent, the mouse-dog mixtures significantly less. Cells of the dog respond significantly in the allogeneic combination and show only a weak response to mouse stimulating cells. In the dog-human combinations there is a response equivalent to or greater than that in the allogeneic mixture. The low response to the mouse stimulating cells will be discussed below.

In the second experiment the human cells respond only very weakly in the xenogeneic mixtures. The dog responding cells show a pattern similar to that in experiment I. The mouse responding cells proliferate more extensively in both of the xenogeneic combinations than in the allogeneic one.

We have done a total of nine experiments using xenogeneic and allogeneic

combinations. In some of these experiments cells of only two species (with at least two members of each species) were included, in others responding cells of two species and stimulating cells of five were used. In all we have tested a total of 158 xenogeneic combinations and compared them with 34 allogeneic ones. The over-all results are given in Table II. In this table the counts per minute in xenogeneic responses are given as a percentage of the counts per minute in the allogeneic ones. This was calculated separately for each responding cell. The data for all the different responding cells of any one species were then pooled. Whereas it would seem that there is on the average less response in xenogeneic combinations, this conclusion must be tempered by technical considerations. As stated above, the cell concentrations, time of culture incubation, and other variables were all chosen to obtain an optimal response in the allogeneic combinations, since this is our usual test mixture; thus the incorporation of tri-

TABLE II  
*Comparison of Allogeneic and Xenogeneic Response in MLC*

Respond- ing cells	Stimulating cells				
	Human	Mouse	Dog	Rat	Rabbit
Human	100 [14]	20.7 (2.7-74.2)* [28]‡	35.5 (2.0-108.3) [22]	45.6 (13.0-93.2) [8]	83.0 (33.0-129.5) [8]
Mouse	118.9 (28.3-305.3) [28]	100 [14]	108.8 (18.0-264.1) [24]	85.9 (42.3-151.9) [8]	101.8 (12.5-303.0) [8]
Dog	103.0 (15.0-240.8) [12]	16.1 (2.4-69.3) [12]	100 [6]		

\* The average response in the xenogeneic mixtures expressed as a percentage of the allogeneic response. The range is given in parentheses.

‡ Number of combinations tested is given in brackets.

tiated thymidine into the xenogeneic mixtures may very well be an underestimate of the amount of stimulation which one might obtain. The quantitative importance of this consideration is not clear; cases of very low stimulation in some of the xenogeneic mixtures may be explained on these grounds.

On the basis of family studies in man, Amos and Bach (10) suggested that stimulation in the MLC test may reflect differences at genetic loci linked to but separate from the *HL-A* loci which are serologically detected. This suggestion has received strong support from more extensive family studies in man (11, 12) as well as from studies in the mouse. We have obtained evidence that differences associated with the *H-2* genetic region, which lead to skin graft rejection but cannot be detected serologically by the usual methods, can result in MLC activation (2). These findings suggest an increased complexity of this genetic region as it relates to MLC activation and leave uncertain the exact nature of the stimulus. Such uncertainty makes even more difficult speculation concerning

the present findings, for instance as they relate to the high frequency of initially responding units in MLC (13, 14).

In view of the findings presented in this paper, the lesser graft-*versus*-host reaction in vivo may not be simply a reflection of a lesser ability to recognize foreignness in xenogeneic combinations, but may have to do with other factors which influence the pathogenesis of graft-*versus*-host reactions in vivo such as inhibition of proliferation of responding cells by the xenogeneic environment.

Wilson and Fox (15) have observed that cells of germfree animals cannot respond in xenogeneic mixtures and have suggested that xenogeneic response may be due to prior sensitization by cross-reacting antigens. An alternate explanation would be that the lack of response in the germfree animals may have been due to the very weak stimulation they observe in xenogeneic combinations in general (6).

#### SUMMARY

The mixed lymphocyte culture (MLC) test has been regarded as an in vitro model of the recognition or sensitization phase of the homograft or graft-*versus*-host reaction. It has been suggested that the graft-*versus*-host response in vivo is less in xenogeneic combinations than in allogeneic ones and that there is a similar quantitative relationship in MLC responses. Given the above interpretation of the MLC test, this could suggest that the lesser reactivity in xenogeneic combinations may be due to a lesser recognition of the stimulus. We have done nine experiments testing allogeneic and xenogeneic combinations in MLC, largely in combinatorial fashion. The results indicate that the response in xenogeneic MLC may be as great as that in allogeneic MLC and that, as in different allogeneic mixtures, there is great variation in the extent to which xenogeneic mixtures may respond.

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