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Genetics

Odortypes determined by the major histocompatibility complex in germfree mice

(chemosensory communication/olfaction/behavior)

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ABSTRACT **The major histocompatibility complex (MHC) is the prime but not exclusive determinant of genetically specific constitutive body odors, termed odortypes, represented strongly in urine of the mouse. Perception of MHC-determined odortypes influences reproductive behavior in the contexts of mate choice and maintenance of early pregnancy, tending to favor the propagation of one MHC type over another. How MHC genotype determines MHC odortype is unknown. One possible explanation is that differential odorants are generated by populations of commensal microorganisms whose composition is somehow geared to MHC diversity. This hypothesis was tested in the Y-maze system in which mice are trained to distinguish the urinary odors of MHC-congenic mice. First, it was shown that mice could readily be trained to distinguish the urines of germfree MHC-congenic mice. Second, it was shown that mice trained to distinguish the urines of conventionally maintained MHC-congenic mice could as readily distinguish the urines of germfree MHC-congenic mice. These results imply that MHC-determined odortypes do not depend on odorants generated by microorganisms.**

Perception of genetically varied constitutive body odors ("odortypes") influences the mating preferences of male mice and the maintenance of early pregnancy in females (1). The major histocompatibility complex (H-2) is paramount in odortype determination—mutation of a single class I H-2 gene is sufficient to cause a change in odortype (2, 3)—but other, unidentified autosomal genes, and both sex chromosomes, also contribute to odortype (1, 4). Use of a Y-maze test system (5, 6) greatly facilitates the definition of odortypes.

The manner in which genotype determines odortype is unknown. One hypothesis, among other exclusively intrinsic explanations, is that the many anatomical and physiological variations ascribed to H-2 diversity (for refs. see ref. 7) entail highly idiosyncratic profiles of excreted odorous metabolites, notably in urine, which is the prime source of odortypes, giving rise to compound odors whose specificity depends on the ratios of constituent odorants rather than on chemical differences (8). But doubtless several modes of genotypical odorant variation may jointly contribute to odortype.

A hypothesis that is not exclusively intrinsic invokes the involvement of commensal microorganisms, a notable source of potent odorants, whose identity might in some manner be geared to H-2 diversity. Provisional support for this possibility comes from a report that in a "habituation/dishabituation" test system, based on assessment of interest paid to alternative familiar vs. unfamiliar samples of urine, rats distinguished the scents of congenic rats differing genetically only at the major histocompatibility complex (9, 10), but not if the urine donors of the same congenic strains were germfree (GF) (11).

The following studies concern the expression of distinctive H-2-determined odortypes by GF congenic mice differing genetically only at the H-2 locus and the relation of these odortypes to those expressed by conventionally maintained mice of the same congenic strains.

MATERIALS AND METHODS

The Y-Maze. The design and operation of the Y-maze used in studying odortypes are detailed elsewhere (5, 6). The two arms of the maze were scented by air currents conducted through chambers containing urine (B6 vs. B6-H-2^k) exposed in Petri dishes.

Rewarded Trials. Mice were trained by water deprivation for 23 hr followed by reward with a drop of water, dispensed mechanically, for entering the arm scented by B6 urine (one group of trained mice) or by B6-H-2^k urine (second group of trained mice). Each training or testing session comprised up to 48 consecutive trials, uniformly timed by raising and lowering gates. Different urine samples from the urine donor panels were provided for each trial (so that the same pair of alternative samples was seldom encountered twice in one session). Assignment of sample pairs to left and right arms was determined by a series of random numbers.

Unrewarded Trials. Once a significant concordance score of around 80% or more was achieved in training, testing was continued as before but without reward for correct choice in every fourth trial. This accustoms the mice to periodic omission of reward, preparatory to generalization trials (see below). Concordance in such interspersed unrewarded trials should not substantially differ from concordance in rewarded trials.

Generalization (Transfer of Training). Testing was continued as described above, but samples from the interspersed unrewarded trials were now supplied from new panels of B6 and B6-H-2^k urine donors, which the trained mice had not previously encountered. Since these new interspersed samples were uniformly unrewarded, these could be coded and blind-tested, and concordance could be determined *post hoc*. Generalization, meaning significant concordance, generally not substantially different from that achieved in training, has the following implications: First, it rules out various experimental artifacts such as inadvertent prompting by the operators of the maze. Second, since there can be no new learning without reward, generalization confirms that the distinction in both cases relates to H-2 disparity rather than to nongenetic and adventitious differences in odor that conceivably might distinguish one panel of mice from another [in line with evidence that mice cannot distinguish panels of genotypically identical urine donors (4, 6)].

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Abbreviations: GF, germfree; CV, conventionally maintained.

Table 1. Age-matched male mice providing urine for test of H-2-determined odortype

Mice				
	No.			
Type	B6	B6-H-2^k	Collection and storage of urine	Use
Monell conventional*	25–40	25–40	Abdominal pressure; individual urine samples frozen immediately	Training Rewarded trials Unrewarded trials
SKI SPF†	20	20	Mice placed in metabolic cages for <18 hr; urine frozen immediately after collection	Generalization
Taconic GF-I‡	6	9	Mice placed in metabolic cages inside isolators; urine collected over 24 hr then removed from isolators and frozen	Training Rewarded trials Unrewarded trials
Taconic GF-II‡	12	14	As for Taconic GF-I	Generalization

*These mice were originally obtained from SKI and were bred and maintained at Monell under uniform conditions. They supported a wide range of commensal flora. They were individually numbered so that they could be used in rotation to provide different sample pairs for each trial.

†Specific pathogen-free (SPF) mice were supplied to Taconic Farms from SKI for use in deriving the GF panels. Non-caesarean-derived offspring of these mice were transferred to Monell where they were housed in individually ventilated cages. They were provided the same diet and bedding as the Taconic GF (GF-I and GF-II) mice, but it is likely that they were exposed at Monell to the same pathogens as Monell conventional (see above).

‡Offspring of the SPF mice described above were delivered by caesarean section under sterile conditions and were fostered on lactating GF Swiss–Webster females housed in sterile isolators. At weaning, GF donors were placed in same-sex groups and reared for 6 weeks. At this time, urine collections from the male mice were initiated.

Sources of Odors. The urine donor panels are described in Table 1. For each day's trials, freshly defrosted urine samples were brought to room temperature and were assigned to the left or right odor boxes of the Y-maze according to a series of random numbers. A given combination of urines of individually numbered mice was not used more than once on any testing day. To confirm that urine of the GF mice ("Taconic GF-I" and "Taconic GF-II"; see Table 1) had not become contaminated, samples of freshly defrosted urine and urine that had been used in 6 hr of Y-maze testing were cultured for bacteria and molds. All samples were negative.

Derivation and Surveillance of GF Mice. GF B6 and B6-H-2^k mice were derived at Taconic Farms from breeders originally obtained from the Sloan–Kettering Institute (SKI) according to standard procedures. Isolators containing foster mothers were tested 10 and 4 weeks before the GF derivations to confirm the absence of aerobic and anaerobic bacteria. Upon caesarean delivery, placental samples were examined for *Mycoplasma* and *Pasteurella* contamination. Four to six weeks postderivation, sentinel mice from isolators housing the caesarean-derived mice were tested and found free of endo- and ectoparasites, bacterial, protozoan, mycoplasmal, and adventitious viral pathogens. Thereafter, isolator swabs were obtained weekly and tested for aerobic and anaerobic bacteria and molds. All isolators containing urine donors used in this study were negative.

RESULTS

Study I. The purpose was to determine whether mice could be trained to distinguish the urine of GF B6 (H-2^b) males from the urine of GF B6-H-2^k males. Although urines of both males and females are sources of H-2-determined odors, male urine is generally somewhat more potent in this respect in the Y-maze test system (6) and also obviates consideration of estrous cycles, hence the use of male urine donors in the present studies.

Neither the sex nor the genotype of mice chosen for training in the Y-maze, nor whether their H-2 types conform to that of either of the alternative urine donors whose H-2 odortypes are to be distinguished, nor which of the alternative urine sources is chosen for reward, have been significant factors influencing ease of training or degree of proficiency attained in training (2, 6). In the present study, two B6-H-2^k mice (one male and one female) were rewarded in training for selecting the odor of B6 urine as opposed to B6-H-2^k urine, obtained from GF donors (Taconic GF-I); two B6-H-2^k mice (one male and one female) were rewarded in training for the alternative selection, B6-H-2^k urine as opposed to B6 urine, obtained from the same GF donors. Results for these reciprocal training modes were not significantly different and so the data are shown combined, as well as separately, in Table 2.

An overall concordance score of 86% ($P < 0.001$) was attained in rewarded trials, 77% ($P < 0.01$) was attained in

Table 2. Odortypes distinctive of H-2^b and H-2^k genotypes are expressed in the urine of GF mice

Reinforced alternative	B6 and B6-H-2^k urine donor	Test phase	No. of trials	Concordance, %
B6 (vs. B6-H-2^k)	Taconic GF-I	Rewarded	93	86
	Taconic GF-I	Unrewarded	18	72
	Taconic GF-II	Generalization	22	86
B6-H-2^k (vs. B6)	Taconic GF-I	Rewarded	77	87
	Taconic GF-I	Unrewarded	12	83
	Taconic GF-II	Generalization	15	80
Total	Taconic GF-I	Rewarded	170	86*
	Taconic GF-I	Unrewarded	30	77†
	Taconic GF-II	Generalization	37	84*

*$P < 0.001$.
†$P < 0.01$.

Table 3. Odortypes distinctive of H-2^b and H-2^k genotypes are similar in CV and GF mice

Reinforced alternative	B6 and B6-H-2^k urine donor	Test phase	No. of trials	Concordance, %
B6 (vs. B6-H-2^k)	Monell conventional	Rewarded	307	86
	Monell conventional	Unrewarded	42	90
	SKI SPF	Generalization	41	76
	Taconic GF-II	Generalization	41	80
B6-H-2^k (vs. B6)	Monell conventional	Rewarded	353	77
	Monell conventional	Unrewarded	42	83
	SKI SPF	Generalization	39	62
	Taconic GF-II	Generalization	41	71
Total	Monell conventional	Rewarded	660	81*
	Monell conventional	Unrewarded	84	87*
	SKI SPF	Generalization	80	69†
	Taconic GF-II	Generalization	82	76*

SPF, specific pathogen free.
*$P < 0.001$.
†$P < 0.01$.

interspersed unrewarded trials (which accustom the trained mice to periodic withholding of reward for concordant choice), and 84% ($P < 0.001$) was attained in interspersed uniformly unrewarded blind trials of coded urine samples from duplicate panels of B6 and B6-H-2^k GF donors (Taconic GF-II) not previously encountered by the trained mice (generalization).

Thus, odortypes distinctive of H-2^b and H-2^k genotypes are expressed in the urine of GF mice.

Study II. The purpose of this second study was to determine whether there is a distinctive difference between H-2-determined odortypes expressed by GF mice (demonstrated in study I above) and H-2-determined odortypes expressed by conventionally maintained (CV) mice.

Three B6 and two B6-H-2^k males were rewarded in training for selecting the odor of B6 urine, as opposed to B6-H-2^k urine, obtained from Monell CV mice. Three B6 male mice and three B6-H-2^k mice (two males and one female) were rewarded in training for the alternative selection, B6-H-2^k urine as opposed to B6 urine, obtained from the same Monell CV donors. Results for these alternative training modes were not significantly different and so the data are shown combined, as well as separately, in Table 3.

A combined concordance score of 81% ($P < 0.001$) was attained in training with respect to Monell CV urine donors in rewarded trials, and a score of 87% ($P < 0.001$) was attained in interspersed unrewarded trials.

Combined concordance was 69% ($P < 0.01$) for interspersed, uniformly unrewarded, blind trials of coded urine samples from SKI specific pathogen-free donors not previously encountered (generalization). [The difference in concordance scores (87% vs. 69%) is significant ($P < 0.01$) and may be attributable to differences in environment, diet, or husbandry between Monell, SKI, and Taconic.]

The combined concordance score for interspersed, uniformly unrewarded, blind trials of coded urine samples from GF donors not previously encountered (Taconic GF-II; generalization) was 76% ($P < 0.001$), signifying that the GF state entails no distinctive alteration in the H-2-determined odortype expressed by CV mice, as judged by proficiency of chemosensory distinction in the Y-maze.

DISCUSSION

Since mice can readily be trained to distinguish the odor of GF H-2^b mice from the odor of otherwise genetically identical (congenic) GF H-2^k mice, and since mice trained to distinguish between conventionally maintained H-2^b and H-2^k congenic mice can as readily distinguish between GF H-2^b and H-2^k congenic mice without training (generalization), it is clear that H-2-determined odortypes do not depend on commensal microorganisms.

The Y-maze test system was chosen because of its simplicity, as compared with the two other test systems that reveal sensory recognition of odortypes—namely, mating preference favoring a nonfamilial H-2 type [a result of familial imprinting (12)], and the raised incidence of blocked pregnancy in females sensing a non-stud H-2 type. However, since all distinctions of H-2-determined odortypes identified in the Y-maze have been shown to influence these systems also, to the extent that these have been tested, it is unlikely that odortypes of GF mice would be exceptional in this regard.

Also, the fact that mice trained to distinguish between H-2^b and H-2^k congenic mice maintained conventionally at one location (Monell) could as readily distinguish between H-2^b and H-2^k congenic mice conventionally maintained elsewhere (Taconic Farms) without training (generalization) adds to the general experience that the constitution of H-2-determined odortypes is not substantially affected by details of environment and husbandry that must inevitably vary when similar mouse colonies are maintained in different locations.

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