

THE HEMATOPOIETIC SYSTEM IS A SOURCE OF
ODORANTS THAT DISTINGUISH MAJOR
HISTOCOMPATIBILITY TYPES

BY KUNIO YAMAZAKI,* GARY K. BEAUCHAMP,* LEWIS THOMAS,‡ AND
EDWARD A. BOYSE‡

*From the *Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104; and the*

‡Memorial Sloan-Kettering Cancer Center, New York, 10021

The olfactory sensing of major histocompatibility types among mice is evident in H-2-associated mating preferences (1–4), in the successful training of male and female mice to distinguish the scents of major histocompatibility complex (MHC)-congenic mice in a Y maze (5–7), and in the raised incidence of pregnancy block in females exposed to the scent of alien males whose H-2 type differs from that of the mate (8). The nature of the MHC-determined odorants perceived by the responding mouse is unknown; possible agencies range from odorous derivatives of MHC products themselves to quantitative differences in output of odorous metabolites reflecting developmental variation geared to MHC polymorphism (9–11). Nor is it known which cells or tissues contribute to the odorant profile.

Since urine is the only material tested that equals the intact mouse as a source of MHC-related odorants, one question that arises is whether the odorants are mostly made by the kidney, or merely concentrated there. We have investigated these alternatives by determining whether radiation chimeras, made by reconstituting lethally-irradiated inbred mice with bone marrow of MHC-congenic (hemiallogeneic) F₁ hybrid donors, acquire a scent typical of the MHC haplotype thereby introduced. If that were so, then cells of the hematopoietic system must contribute to the MHC-related odorant profile. Hybrid donors were used to obviate graft-vs.-host disease.

Materials and Methods

Table I shows the constitution and designations of the radiation chimeras and the number of chimeras in each urine donor panel. The mice of paired panels were matched for age and individually numbered by ear punch for use in rotation to provide different sample pairs for each trial run. 4–12 wk after irradiation, urines were collected from the chimeras for testing in the transfer of training phase (see below), and frozen until needed; these urine samples were coded for blind testing, and a new pair of sample donors was used for each trial.

The design and operation of the Y maze are described in detail elsewhere (5). Briefly, air is conducted through two odor chambers, containing urine samples exposed in petri dishes, to the two arms of the maze. Gates are raised and lowered in timed sequence to

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TABLE I
Constitution of Radiation Chimeras

Set	Reconstituting male cell donors*	Lethally irradiated male recipients [†]	Designation of chimeras	Chimeras in each panel
1	B6 (B6 × B6-H-2 ^k)F ₁	B6	B6/B6	22
		B6	bk/B6 [‡]	16
2	(B10.A × B6)F ₁ (B10.S × B6)F ₁	B6	ab/B6 [‡]	19
		B6	sb/B6 [‡]	19

* Providing 4.5–7.5 × 10⁷ bone marrow and spleen cells per recipient, intravenously.

[†] ¹³⁷Cs gamma radiation source; 940 rad in set 1, and 990 rad in set 2.

[‡] Checked 5–11 wk after recovery by cytotoxicity test for H-2 of donor type on >95% of cells from an excised lymph node (b, H-2^b; k, H-2^k; a, H-2^a; s, H-2^s).

TABLE II
Performance in Rewarded Trials of B6 vs. (B6 × B6-H-2^k)F₁ (bk) Urine Donor Panels and Transfer of Training (Unrewarded) to B6/B6 vs. bk/B6 Chimeric Donor Panels

Test phase	Urine donor panels	Number of trials	Percent concordance, and significance
Training with reward	B6 vs. bk mice	562	79 (<i>p</i> < 0.001)
Transfer to chimeras* (interspersed trials of coded samples without reward)	B6/B6 vs. bk/B6 chimeras	70	80 (<i>p</i> < 0.001)

The trained mice comprised a B6 male and female, a B10 male, a B6-H-2^k male, a B10.S male and female, and a (BALB/c × B6)F₁ male.

* See Table I for constitution of chimera.

permit the training or testing of each mouse in a series of up to 48 consecutive runs, the samples being changed for each run, and left-right placement determined by random numbers. The reward is a drop of water, the mouse having been deprived of water for 23 h beforehand. The water dispenser in each arm of the maze is guarded by a fence, which is raised only if the mouse's choice is concordant with training (correct). To permit testing of new samples without reward, thus obviating the possibility that new incidental or genetically unrelated cues are being learned and responded to, the transfer of training procedure (6) was employed in testing the chimeras. Transfer of training is conducted with blind testing of coded samples, which is possible because no reward is called for. To maintain reinforcement (concordant response to the learned scent) the unrewarded coded samples from the chimeric mouse panels were interspersed with concurrent, rewarded testing of samples from the familiar training panels.

Results

Set 1 comprises two series of experiments in which mice were first trained by reinforcement for (B6 × B6-H-2^k) in preference to B6. The data were similar in the two series and have been combined in Table II. The upper part of Table II shows concordance of 79% (*p* < 0.001) for 562 performance trials in which reward was withheld on every eighth trial, in preparation for transfer of training. The lower part of Table II shows 80% concordance (*p* < 0.001) for the 70 transfer of training (unrewarded) trials, in which coded sample pairs from the (B6 × B6-H-2^k)F₁/B6 and B6/B6 control chimera panels were substituted in every eighth run in regular performance trials, as above. Clearly, the introduction of hematopoietic cells whose H-2 type corresponds to the H-2 type for which discrimination was learned in training suffices to confer a scent characteristic of that H-2 type.

TABLE III
Performance in Rewarded Trials of (B10.A × B6)F₁ vs. (B10.S × B6)F₁ Urine Donor Panels (ab vs. sb), and Transfer of Training to Corresponding Chimeric Donor Panels

Test phase	Urine donor panels	Number of trials	Percent concordance, and significance
Training with reward	ab vs. sb mice	342	81 ($p < 0.001$)
Transfer to chimeras* (interspersed trials of coded samples without reward)	ab/B6 vs. sb/B6 chimeras	54	69 ($p < 0.01$)

The trained mice comprised (B6 × B6-H-2^b)F₁ hybrids and typed (B6 × B6-H-2^b)F₂ segregants (H-2^b/H-2^b or H-2^b/H-2^s), some male and some female, some reinforced for *ab* (H-2^s/H-2^b), and some for *sb* (H-2^s/H-2^b); the data are combined because performance did not significantly differ among these eight trained mice.

* See Table I for constitution of chimera.

In set 2, the possibility of some covert difference entailed by the constitution of syngeneic (control) chimeras, as compared with hemiallogeneic chimeras (as was the case in set 1), was evaluated by testing a pair of hemiallogeneic chimera panels. The subject mice were first trained to distinguish between congenic F₁ hybrid mice (H-2^a/H-2^b vs. H-2^s/H-2^b), and were then tested by transfer of training to (H-2^a/H-2^b)/B6 chimeras vs. (H-2^s/H-2^b)/B6 chimeras. Otherwise the experimental design was the same as in set 1. As Table III shows, the concordance in 342 rewarded performance trials was 81% ($p < 0.001$) and 69% ($p < 0.01$) in the 54 interspersed unrewarded transfer of training trials of coded samples from corresponding chimeric mice; both, in this case, reconstituted with hemiallogeneic donor cells.

Discussion

The data indicate that cells of the hematopoietic systems contribute sufficiently to the MHC-related odorant profile to permit the distinction of one mouse from another by scent. It remains to be seen what other cells or tissues also may contribute. In the chimeras studied, the host's MHC type was the same as one of the hybrid donor's haplotypes. Such chimeras can give no information on the contribution of nonhematopoietic cells to the odorant profile. That would require fully allogeneic H-2-congenic donors, which we considered unsuitable for studying retention of odorant properties typical of the recipient because graft-vs.-host disease, whether obvious or not, seemed an unacceptable complication.

Summary

Radiation chimeras were made by restoring lethally irradiated inbred mice with bone marrow cells of F₁ hybrid mice of crosses between that inbred strain and an H-2-congenic strain. The urine of these chimeras was tested by the Y maze method, and shown to have acquired a scent indicative of the reconstituting donors' H-2 type. Thus, cells of the hematopoietic system contribute to the H-2-related odorant properties that enable mice to distinguish one another according to their H-2 types.

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