FACTORES DE RECONOCIMIENTO ENTRE CELULAS INMUNOCOMPETENTES Y SUS LIGANDOS.

I. Efecto de la Valencia del Ligando.

II. Receptores Solubles (Anticuerpos).

III. Interacciones con Células Tumorales y Enmascaramiento Antigénico.

T E S I S

Que Presenta:

ESTEBAN CELIS FLAM

Para Obtener el Grado de:

DOCTOR EN CIENCIAS

Unidad Académica de los Ciclos Profesional y de Postgrado,
Colegio de Ciencias y Humanidades.

Instituto de Investigaciones Biomédicas,
Universidad Nacional Autónoma de México.

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CONTENIDO:

6.- CELIS ESTEBAN, Chang, T.W., and Eisen, H.N.: Cyclical Changes in Susceptibility of a Myeloma Tumor (LPC-1) to Immune Destruction III. Periodic Production of a Cell Surface Glycoprotein and Changes in Reactivity with Cytotoxic T Cells and Anti-H-2^d Sera. Journal of Immunology 122, 2245-2250 (1979).

7.- CELIS ESTEBAN, and Eisen H.N.: Interactions Between a Novel Cell Surface Glycoprotein and the H-2K and H-2D Antigens on Myeloma Tumor Cells. European Journal of Immunology (en prensa).

8.- CELIS ESTEBAN: Cellular Recognition in Tumor Immunology: Tumor Resistance to Immune Destruction by Cytotoxic T Lymphocytes. en "Molecules, Parasites and Cells", editado por Larralde, Ortiz, Willms y Sela. Academic Press, New York (en prensa).

IV. DISCUSION GENERAL

V. BIBLIOGRAFIA GENERAL

INTRODUCCION GENERAL

El trabajo que aquí se presenta se centra en el estudio de los mecanismos involucrados en el reconocimiento de antígenos (Ags)* por las células inmunocompetentes. Ehrlich fué el primero en proponer la existencia de moléculas en la superficie de los linfocitos con función de receptores para Ags (1). En la actualidad es un hecho ya aceptado que los linfocitos tanto B (derivados del bazo) como T (o del timo) poseen en sus superficies receptores específicos para Ags (2-4). Mientras que el receptor para Ags de los linfocitos B parece ser una inmunoglobulina muy similar a los anticuerpos (Acs) que se encuentran en la circulación sanguínea (5, 6), la naturaleza bioquímica del receptor de los linfocitos T es aun incierta (4, 7, 8). La especificidad de la reacción de los receptores de los linfocitos B con sus Ags, se espera sea muy similar a aquella que se presenta en la reacción Ag - Ac (9). Sin embargo, la reacción de los linfocitos T con sus ligandos (generalmente otras células) no es del todo tan simple, ya que se ha observado que los productos del complejo génico mayor de histocompatibilidad (CMH), llamado H-2 en el ratón y HLA en el humano, juegan un papel determinante en dicho reconocimiento (4). Es decir, el linfocito T reconoce al Ag en contexto de los productos del CMH que se expresan en la superficie de las células con las cuales interaccionan (células blanco).

Es de esperarse que en ambos casos, con linfocitos B y T, factores tales como la naturaleza fisico-química del ligando, ya

sea éste soluble (v.g., proteínas y carbohidratos) o insoluble (agregados moleculares, bacterias y células blanco), su valencia, móleculas que compitan con la reacción (como Acs) o que enmascaren los sitios antigénicos del ligando y por último el microambiente donde se lleve a cabo la reacción jueguen un papel primordial en dicha reacción receptor-ligando.

La serie de artículos aquí presentados son un intento de estudiar algunos de los factores que pueden determinar y regular la reacción del receptor de las células inmunocompetentes con sus ligandos. Inicialmente se elaboró un modelo bioquímico simulando a los linfocitos B y sus receptores por medio de microesferas de agarosa (Sepharose o Sefarosa) acopladas con Acs purificados. Utilizando dicho modelo y Ags solubles radioactivos, con distinto número de determinantes antigénicos de la misma especificidad por molécula (valencia) se estudió el efecto que ejercía la valencia antigénica sobre la constante aparente de afinidad de la reacción receptor-ligando (10). Se encontró primero, que la constante aparente de afinidad (Ka) es proporcional a la valencia del antígeno, y segundo, la existencia de un trazo ascendente en las gráficas de Scatchard (11), también relacionado con la valencia antigénica que parece indicar cierta cooperatividad entre los sitios activos de los receptores (10).

Utilizando el mismo modelo bioquímico, se estudió además el efecto que receptores solubles o Acs podrían tener en la reacción del receptor celular simulado con sus Ags. Se observó que los Acs

ejercían un papel regulatorio en la reacción del ligando soluble con los receptores celulares: cuando el ligando era polivalente pequeñas cantidades de Acs actuaron sinergicamente en la reacción, incrementándose la cantidad total de ligando que se fijó a los receptores celulares (12). Sin embargo, cantidades mas grandes de Acs actuaron competitivamente inhibiendo la reacción receptor-ligando (12). Cuando se utilizaron ligandos monovalentes en la reacción con receptores y Acs, unicamente se observó una disminución de la fijación del ligando a los receptores celulares (12). En estos experimentos se observó además una ventaja significativa (de más de 100 veces) de los receptores sobre los Acs en su capacidad de fijar ligandos monor y polivalentes. Es decir, que para inhibir la fijación de ligando, por cada sitio activo de receptor, se necesitaron mas de 100 sitios activos de Acs (12). Finalmente, en este trabajo se propone un modelo teórico simulado en una computadora donde se ilustra como los Acs pueden ejercer un papel regulatorio durante el curso de una respuesta inmune (12).

Utilizando un modelo experimental distinto, esta vez con células vivas, se procedió a estudiar la reacción de reconocimiento de los linfocitos T con sus células blanco. Llevando a cabo observaciones minuciosas al microscopio óptico y al electrónico de las interacciones de los linfocitos T con sus células blanco adheridas a cajas de Petri fué evidente que el proceso de reconocimiento es muy dinámico y complejo. Se observó que los linfocitos T cuando se encuentran "activados", recorren toda la superficie de las células blanco con movimientos amiboides muy rápidos (13). Esta exploración de

la estructura antigénica de las células blanco por los linfocitos T parece ser inespecífica, ya que se presentó aún cuando se utilizaron células blanco que carecen del Ag al cual las células T se encontraban dirigidas (13).

Como se mencionó anteriormente, el reconocimiento de los Ags por los linfocitos T se haya supeditado a la expresión de algunos de los productos del CMH. La importancia de los Ags H-2K y H-2D del CMH del ratón en la interacción de células tumorales con linfocitos T citotóxicos (LTCs) es evidente por los resultados obtenidos en los demás trabajos presentados (14-18). Específicamente se encontró que algunos tumores de mieloma de ratón se vuelven resistentes a la actividad citolítica de los LTCs enmascarando sus moléculas H-2K y H-2D por medio de una glicoproteína que los mismos miekomas sintetizan y expresan a la superficie celular (14-16). Al parecer, esta glicoproteína de 160,000 daltones (gp160) interacciona de una manera preferencial con H-2K y H-2D afectando relativamente poco el despliegue de otros Ags en la superficie de estas células tumorales (17, 18).

LA EVASION DE LOS TUMORES A LA RESPUESTA INMUNE

Esteban Celis Flam

INTRODUCCION

Destrucción de Tumores por el Aparato Inmunocompetente. Los tumores malignos por lo general se caracterizan por además de su crecimiento y multiplicación incontrolablemente acelerados y su capacidad de diseminación (metástasis), por tener en su superficie celular estructuras "alteradas" que el organismo reconoce como extrañas o antígenos (Ags). La naturaleza de estas estructuras en la superficie de las células tumorales o Ags tumorales es muy diversa: los tumores experimentales inducidos por virus tienden a expresar Ags similares los cuales por lo general son producto del genoma viral. Por otro lado, los tumores inducidos por agentes químicos expresan Ags tumorales muy variados, algunos de ellos - probablemente de origen fetal y otros posibles productos de mutaciones - inducidas por las substancias químicas tumorigénicas (1-3).

En condiciones experimentales se ha podido demostrar que algunos de los productos de la respuesta inmune a los Ags tumorales tales como los anticuerpos (Acs) y células de diversos tipos como lo son linfocitos y los macrófagos, son capaces de rechazar y destruir tumores transplantables (4-6). Dentro de las reacciones inmunes mediadas por células con actividad anti-tumoral, la mas importante parece ser la producida -- por los linfocitos citotóxicos derivados del timo. Estas células, los - linfocitos T citotóxicos (LTCs) son también las encargadas de combatir - algunas infecciones virales y de rechazar transplantes de tejidos. Aunque el mecanismo de lisis de los LTCs todavía es desconocido, se sabe --

que para que estas células reconozcan y maten a sus células blanco (célu la tumoral, infectada por virus o transplantada), es necesario que estas últimas expongan en su superficie algunos de los productos del complejo génico mayor de histocompatibilidad. En el caso de células tumorales y células infectadas por virus, es necesario también que éstas expresen en su superficie al Ag correspondiente ya sea tumoral o viral para que los LTCs puedan interaccionar con ellas (7).

El gran interés que ha causado el complejo génico mayor de - histocompatibilidad dentro de la inmunología durante los últimos años es en gran parte debido al número creciente de funciones ligadas a la res-- puesta inmune que le han sido atribuídas a éste (8-11). Este complejo - llamado H-2 en el ratón y HLA en el humano es un conglomerado de genes - que determinan fenómenos inmunes tales como la regulación de respuestas de Acs a determinados Ags y la expresión de moléculas de superficie celu lar involucradas con interacciones celulares complejas entre ellas las - de los LTCs con sus células blanco. Los complejos H-2 y HLA codifican - por lo menos cada uno, dos moléculas o aloantígenos que se encuentran -- presentes en la mayoría de las células del organismo y que intervienen - en la reacción con los LTCs. Estas moléculas que han sido estudiadas -- con mucha intensidad, se encuentran codificadas por genes extremadamente polimórficos, es decir que existe un gran número de formas alélicas dentro de una misma especie (8-10). Los aloantígenos H-2K y H-2D en el ratón y HLA-A y HLA-B en el hombre han sido caracterizados como glicopro-- teínas integrales de membrana de aproximadamente 45,000 daltons de peso

molecular (12,13).

Se desconoce si los LTCs poseen en su superficie dos receptores distintos, uno para el Ag de superficie celular "X" (tumoral, viral, Ags menores de histocompatibilidad) y otro para los aloantígenos H-2 o HLA, o si estas células citotóxicas tienen únicamente un solo receptor que reconoce y reacciona con una asociación de los dos Ags ("X" y H-2 o HLA) en la superficie de las células blanco. Lo que si se sabe es que los LTCs interaccionan solamente (o en mayor grado) con las células blanco que expresan los mismos Ags H-2 (o HLA), es decir la misma forma alélica, presentes en las células que inicialmente estimularon la reacción inmune celular. Este fenómeno conocido como "restricción por H-2" (o por HLA), es bastante general y ha sido descrito en sistemas donde los LTCs se encuentran dirijidos en contra de Ags virales (14), tumorales (15-17), Ags menores de histocompatibilidad (18), o Ags inducidos por medios químicos (19,20). Es claro que el papel que juegan los Ags H-2 y HLA es esencial para el reconocimiento celular por los LTCs, sin los alcantígenos aunque se encuentre presente el Ag "X" en la superficie de la célula blanco, no hay función del LTC. Por lo tanto, se antoja que un posible papel de los aloantígenos H-2K y H-2D (o HLA-A y HLA-B) es que sean estructuras de "autoreconocimiento"; existe evidencia de que los LTCs aprenden del timo durante su proceso de diferenciación esta propiedad de autoreconocimiento (21,22). Además, ya que la función de los LTCs supone la interacción directa con la superficie de la célula blanco, la participación de los aloantígenos H-2 y HLA ayudaría a evitar que Ags

"X" en solución como lo podrían ser partículas virales o Ag tumorales -- que han sido liberados al medio, compitan con los Ags en la superficie - de las células blanco y los LTCs sean desviados de su fin, lo que sucedería si los Ags "X" fuesen reconocidos por si solos por los LTCs.-

Resistencia Inmune a Tumores. Como se mencionó con anterioridad, las reacciones inmunes de tipo celular, principalmente las de -- LTCs parecen ser las que determinan el rechazo y la destrucción de tumores transplantables. Un gran número de estudios han demostrado que ratones singénicos (de la misma cepa y con el mismo tipo alélico de H-2) pueden sobrevivir al transplante de un gran número de células tumorales si éstos han sido previamente inmunizados con células irradiadas o atenuadas del mismo tumor (5). Esta capacidad de rechazar al tumor puede ser transferida de un animal a otro de la misma cepa por medio de linfocitos T - (posiblemente LTCs) inmunes y además puede ser medida in vitro al cocultivar células tumorales con células T inmunes, midiendo la inhibición -- del crecimiento tumoral o la muerte de las células tumorales por la liberación de radioisótopos (4,5). Estas observaciones señalan que el aparato inmunocompetente debería ser capaz de detectar y destruir tumores que en algún determinado momento surgen espontáneamente o son inducidos por algún tipo de agente oncogénico (substancias químicas o virus). Ehrlich en 1909 fué el primero en señalar esta teoría de una "vigilancia inmunológica" (23), la cual ha sido más recientemente postulada por Thomas (24), Burnet (25) y otros (26,27). Surge automáticamente la pregunta de que si el organismo posee dicha capacidad de reconocer y destruir a las células

tumorales, ¿porqué en un gran número de casos los tumores se desarrollan, crecen y matan a sus huéspedes?.

Entre los posibles caminos de escape de la vigilancia inmunológica que se pueden señalar se encuentran: 1) Los estados de inmunosupresión en que el individuo puede llegar a encontrarse, facilitándose así el desarrollo del tumor (26); dichos estados de inmunosupresión pueden al parecer ser inducidos por el mismo tumor (28). 2) Los Ags tumorales pueden no ser lo suficientemente fuertes para inducir una respuesta inmune - celular rápida y eficaz que proteja y destruye al tumor antes de que éste mate a su huésped (1,29). 3) El tumor puede crecer con tal rapidez que no da tiempo al organismo para elaborar una respuesta inmune protectora. 4) En sus inicios las células tumorales no expresan Ags en su superfi--- cie, y no es sino hasta mas tarde cuando la masa tumoral se ha desarrollado significativamente que éstos pueden ser detectados, es decir que los - tumores pueden llegar a ser capaces de modular la expresión de algunos de sus Ags en la superficie celular (30,31). 5) Los Ags de las células tumorales, no son accesibles a los LTCs por encontrarse bloqueados por Acs (4) o por proteínas mismas del tumor (49-59). 6) Los tumores se vuelven resistentes a la destrucción por LTCs por ser inmunes al mecanismo (hasta ahora desconocido) que utilizan estas células para destruir a sus células blanco.

A continuación se presentan algunos ejemplos que han aparecido recientemente en la literatura acerca de unos de estos mecanismos de - escape a la vigilancia y destrucción inmunológica utilizados por tumores

de origen murino.

INMUNOSUPRESION Y TUMORES

Aunque el índice de tumores expontáneos en individuos inmunosuprimidos no parece ser significativamente mayor al de los individuos con funciones inmunes normales, si existe claramente una gran diferencia en cuanto al desarrollo de tumores inducidos ya sea por agentes virales o agentes químicos. Ratones timectomizados, desnudos (congénitamente atímicos) o aquellos tratados con suero antilinfocítico son más susceptibles a desarrollar tumores inducidos por virus de polioma (32), virus de leucemia murina (33) o por algunos agentes químicos (34-36) que ratones inmunológicamente normales tratados con los mismos agentes oncogénicos.

Recientemente Berendt y North (28) describen como un sarcoma transplantable de la cepa de ratón BALB/c induce la formación de células con actividad supresora durante su crecimiento en ratones normales, pero no cuando crece en animales timectomizados. Los autores demuestran que estas células son linfocitos T supresores que son capaces de inhibir a células inmunes con actividad antitumoral (también linfocitos T), que logran desarrollarse en los animales timectomizados. La actividad de dichos linfocitos T supresores parece ser bastante específica ya que no interfiere con otras funciones inmune celulares tales como el rechazo de alotransplantes.

EXPRESION Y ACCESIBILIDAD DE LOS ANTIGENOS DE SUPERFICIE EN LAS CELULAS TUMORALES

Como se mencionó en la introducción, la restricción por H-2 dictamina que para que un LTC interaccione y destruya a su célula blanco, esta última necesita expresar en su superficie además de una molécula -- extraña o Ag (en el caso de células tumorales, un Ag tumoral) a los productos H-2K y H-2D del complejo génico mayor de histocompatibilidad. Una de las pruebas mas contundentes de que los alo antígenos H-2 juegan un -- papel esencial en la reacción de lisis de las células tumorales por LTCs son los experimentos realizados con el timoma murino TL. Una sublínea - mutante de dicho tumor llamada TL^- no expresa en su superficie las moléculas H-2K y H-2D, pero sí a la mayoría de los otros Ags presentes en la sublínea normal TL , que también expresa a los Ags H-2. Las células -- TL^- son incapaces de estimular la producción de LTCs o de servir como -- células blanco à LTCs inducidos por las células TL (37-39). Aunque este probablemente no sea un buen ejemplo de un mecanismo de escape de la vigilancia inmunológica ya que en la mayoría de los tumores que se estudian sí se encuentran presentes en la superficie celular los Ags H-2, si constituye un caso donde se hace patente la importancia y necesidad de la expresión de los Ags mayores de histocompatibilidad en la superficie de -- las células tumorales, para que éstas puedan interaccionar con los LTCs. Existen otros trabajos donde se mencionan tumores que al parecer no expresan algunos de sus Ags H-2 y otros más donde las células tumorales -- poseen una forma alterada de éstos. En un linfoma producido en un ratón heterozigoto que debería expresar dos formas alélicas de cada uno de los

aloantígenos H-2 (dos H-2K y dos H-2D) ya que estos Ags se expresan de un modo codominante, una de las formas alélicas de H=2K desaparece de la -- superficie de estas células si se les mantiene en cultivo. Por otro lado, es posible inducir la expresión del aloantígeno perdido si se crecen las células tumorales in vivo (40). Otro caso muy similar ocurre con un linfoma de una cepa pura de ratón donde la molécula H-2K desaparece de la superficie celular si las células se cultivan y reaparece si se transfieren a un ratón mientras que el Ag H-2D se mantiene constante (41). La -- pérdida estable de uno de los aloantígenos H-2, el H-2K en una leucemia expontánea de la cepa AKR de ratón, probablemente debida a una mutación (42) constituye otro ejemplo de la ausencia de una de las moléculas de - H-2 sin que se altere la expresión de la otra (H-2D). En los casos en el que la desaparición del aloantígeno es reversible bajo distintas condiciones de crecimiento, se podría pensar en un fenómeno de modulación antigénica descrito anteriormente para otros Ags de superficie celular (30,31). Algunos tumores pueden también expresar formas alteradas de sus Ags H-2, se han descrito varios tumores murinos que expresan formas alélicas distintas de H-2 (43). Queda la duda de que tan importantes puedan ser estos fenómenos como mecanismos de escape a la vigilancia inmunológica. Por un lado, se necesitaría que todos los Ags H-2 dejaran de expresarse en la superficie de los tumores para que éstos sean inertes con los LTCs. En - cepas puras o individuos homocigotos, situación poco común en la naturaleza, sólo se trata de dos aloantígenos un H-2K y un H-2D, mientras que una célula tumoral en condiciones normales y en individuos silvestres debe expresar cuatro aloantígenos H-2, dos formas alélicas distintas de H-2K y -

dos de H-2D, siendo cada forma alélica determinada por la información -- genética derivada de cada uno de los progenitores del individuo. Pare-- cería poco probable de acuerdo a las observaciones realizadas, que todos los aloantígenos desaparecieran de la superficie celular del tumor a un mismo tiempo, ya fuese por mutación o modulación antigénica. Por otro - lado, si la célula tumoral llegase a expresar una forma alterada o una - forma alélica distinta de los Ags H-2, sería de esperarse que el indivi- duo rechazara al tumor mediante una aloreacción mediada también por LTCs similar a las que determinan los rechazos de alotransplantes de tejidos, a menos de que existiera un fenómeno de inmunosupresión.

Por medios inmunológicos de selección se ha podido demostrar que dentro de una población de células tumorales existen algunas con can- tidades muy pequeñas de todos sus Ags H-2. Esta heterogeneidad en la -- concentración de H-2 sí podría significar una ventaja para que algunas de las células tumorales puedan eludir a los LTCs (44).

Es necesario señalar que la presencia de los Ags de superfi- cie celular no necesariamente implica que éstos sean accesibles a los -- productos de la respuesta inmune, Acs y LTCs. El bloqueo de Ags tumora- les por Acs (Acs bloqueadores), que permite que las células tumorales se vuelvan resistentes a la lisis por LTCs fué descrito hace tiempo por los Hellström (4). Otro tipo de bloqueo de los Ags de superficie de las cé- lulas tumorales es aquel causado por productos de las células tumorales mismas. Un ejemplo de ésto es el extenso trabajo realizado por varios - grupos en el adenocarcinoma mamario TA3 de la cepa A/HeHa de ratón ---

(45-52). Una sublínea de este tumor, la TA3-Ha puede ser transplanteada eficazmente a travez de barreras de histocompatibilidad (en otras cepas de ratón y hasta en ratas) sin ser rechazado, mientras que la otra sub-- línea, la TA3-St sólo crece en la cepa de ratón donde se originó el tumor (45,46). Las células de la sublínea TA3-Ha reaccionan considerablemente en menor grado con Acs anti-H-2 que las células de la sublínea TA3-St, - lo que explica las diferencias en transplantibilidad entre ellas (47,48). Una cuantificación cuidadosa de los Ags H-2 en ambas sublíneas Ha y St -- con células íntegras y con distintas fracciones celulares señala que los Ags H-2 si se encuentran presentes en la superficie de las células Ha en concentraciones similares a las encontradas en la sublínea St, y que por lo tanto de alguna manera éstos se encuentran bloqueados en las células Ha íntegras (49). La presencia de una capa muscinosa en la superficie de -- las células TA3-Ha compuesta principalmente de una glicoproteína llamada "epiglicanina" de 500,000 daltons, rica en ácido siálico, que se encuentra ausente en las células TA3-St sugiere que muy probablemente esta glicopro teína de algún modo enmascara a los Ags mayores de histocompatibilidad -- H-2 haciendo que las células sean inertes con LTCs y Acs anti-H-2 (50,51). Se desconoce si la epiglicanina enmascara también a otros Ags además de - H-2 presentes en la superficie del tumor TA3-Ha y el mecanismo por medio del cual produce dicho enmascaramiento. Parece ser que el ácido siálico no juega por si solo un papel preponderante en dicho bloqueo ya que cuan- do cuidadosamente es removido de la superficie de las células TA3-Ha por medio de un tratamiento con neuraminidasa, éstas siguen reaccionando po-- bremente con los Acs anti-H-2 y pueden sobrevivir en transplantes en hués

pedes alogénicos (52). Por otro lado, existe un reporte en la literatura donde se menciona que el ácido siálico en la superficie de una sublínea - de células L de ratón enmascara un gran número de sitios antigénicos ha-- ciendo que las células sean resistentes a la lisis por Acs y complemento, a menos de que las células sean tratadas previamente con neuraminidasa -- (53).

Otro ejemplo de enmascaramiento de los Ags H-2 que produce -- resistencia a la lisis por LTCs causado por un producto mismo de la célula tumoral es el observado en algunos mielomas de la cepa BALB/c de ratón. Los mielomas LPC-1 y MOPC-315-OPEC se caracterizan por reaccionar pobre-- mente con LTCs y Acs anti-H-2 a menos de que las células, sean tratadas - con algunas proteasas como la tripsina y la quimotripsina (54-57). Estas células de mieloma presentan en su superficie grandes cantidades de una - glicoproteína de 160,000 daltons (gp 160) que es susceptible a la proteólisis por dichas proteasas (56). La glicoproteína gp160 se encuentra compuesta por una sola cadena polipeptídica sintetizada por las células tumorales y no se encuentra presente en la superficie de otros tumores de la misma cepa de ratón que son altamente susceptibles a los LTCs y Acs anti-H-2 (54,56). Estudios recientes parecen señalar que gp160 interacciona preferencialmente con H-2K y H-2D ya que la accesibilidad de otros Ags -- presentes en la superficie de los mielomas LPC-1 y MOPC-315-OPEC no se encuentra significativamente alterada (57).

Una proteína similar a gp160 ha sido descrita por un grupo de investigadores de Japón, se trata de una glicoproteína de 120-160,000 dal

tons presente también en la superficie de un mieloma de la cepa BALB/c. Las células de este mieloma reaccionan muy poco con Acs anti-H-2 a menos de que sean tratadas con tripsina o pronasa, lo cual elimina de su superficie a la glicoproteína bloqueadora (58). A diferencia de gp160, esta glicoproteína parece también bloquear el acceso de otros Ags (además de H-2) a sus Acs (59).

Otros ejemplos de tumores resistentes a LTCs pero donde no se ha podido detectar moléculas bloqueadoras de Ags de superficie son los siguientes: En un trabajo con el mastocitoma P815 de la cepa DBA/2 de ratón se reporta que las células tumorales se vuelven resistentes a la lisis por LTCs dirijidos en contra de los Ags tumorales pero no a LTCs dirijidos en contra de los Ags H-2 únicamente (LTCs alogénicos). Los autores señalan la posibilidad de que los Ags tumorales de P815 puedan encontrarse bloqueados por Acs u otro tipo de molécula, sin embargo no existe prueba de esta hipótesis (60). En otro estudio con el mismo tumor pero en condiciones bastante diferentes, se seleccionaron células del tumor P815 resistentes a LTCs creciendo a dichas células en ratones BALB/c. Aunque ambas cepas de ratón, la DBA/2 y la BALB/c son del mismo tipo alélico de H-2, éstas poseen muchas diferencias menores de histocompatibilidad. Sin embargo, si se extraen las células P815 de los ratones BALB/c antes de que sean rechazadas, éstas muestran cierta resistencia a LTCs sin que la concentración de sus Ags H-2 se encuentre alterada. Los autores de este trabajo explican dicho fenómeno a que posiblemente los ratones BALB/c producen Acs a Ags de P815 (a excepción de H-2) que causan un impedimento estérico en la reacción con LTCs (61). Sin embargo,

no se logran detectar inmunoglobulinas en la superficie de estas células tumorales.

Finalmente, en un trabajo realizado con otro mieloma de la cepa BALB/c, llamado MOPC-315-EL se observa que las células tumorales se hacen resistentes a todo tipo de LTCs conforme se desarrolla en la cavidad peritoneal de ratones BALB/c. En este caso a diferencia de los antes mencionados con mielomas, la cantidad de Ags H-2 no se encuentra disminuida (62,63). Como estos cambios de células sensibles a resistentes también se observan cuando las células crecen en ratones desnudos (congenitamente atímicos) y en cultivo es poco probable que la resistencia a LTCs se deba a Acs anti-Ags tumorales que pudiesen bloquear estéricamente la reacción celular de citotoxicidad. Alternativamente es posible que estas células deban su resistencia a LTCs a cambios en la superficie celular (carga iónica, fluidez de la membrana, etc.) o a que hayan adquirido cierta "inmunidad" al mecanismo (hasta ahora desconocido) lítico de los LTCs.

CONCLUSIONES GENERALES

En el presente trabajo se ha presentado una serie de ejemplos de algunos de los posibles mecanismos que pueden utilizar las células tumorales para evadir a la respuesta inmune. Queda claro que los tumores pueden escapar a la destrucción por el aparato inmunocompetente por medio de dos caminos principales: Primero, desarrollándose en individuos inmunosuprimidos, causando ellos mismos un estado de inmunoparáli-

sis o no estimulando una respuesta inmune al "esconder" sus Ags de su-- perficie. En cualquiera de estos casos, el organismo huésped no logra desarrollar una respuesta inmune en contra del tumor. El segundo camino general de evasión inmune es aquel en que el huésped sí establece una - respuesta inmunológica antitumoral pero las células se hacen resisten-- tes a la destrucción ya sea enmascarando sus Ags de superficie por me-- dio de Acs del mismo huésped (Acs bloqueadores) o con moléculas que las células tumorales sintetizan, o por medio de algún otro cambio que las transforman y vuelven inertes a la acción citotóxica de los LTCs.

Sería bastante simplista y riesgozo el tratar de señalar -- cual de estos mecanismos es el mas importante o el mas común para expli- car el desarrollo de las enfermedades neoplásicas y la ineficacia de la respuesta inmune para combatirlas. Es probable que varios de ellos, - además de otros que faltan por descubrirse sean igualmente responsables. Se antoja pensar que en un mismo padecimiento tumoral, mas de uno de -- los mecanismos señalados se encuentren involucrados, por ejemplo, en el inicio del crecimiento tumoral podría haber un fenomeno de inmunosupre- sión y mas adelante, si el huésped logra superar la parálisis inmunoló- gica, es posible que existiera en enmascaramiento de los Ags de super-- ficie.

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EFFECTS OF THE EXTENT OF DNP SUBSTITUTION ON THE APPARENT AFFINITY CONSTANT AND COOPERATION BETWEEN SITES IN THE REACTIONS OF DINITROPHENYLATED HUMAN SERUM ALBUMIN WITH ANTI-DNP AND ANTI-HSA ANTIBODIES COUPLED TO AGAROSE

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Abstract—Increments in the extent of dinitrophenyl (DNP)* substitution of human serum albumin (HSA) increases the apparent affinity constant of its reaction with anti-DNP antibodies coupled to agarose and decreases that of its reaction with anti-HSA antibodies also fixed to the same solid phase. An ascending limb of the Scatchard plot in the low levels of bound ligand is described and is shown to be also dependent of the degree of DNP substitution of the antigen. Results are discussed as indicative of a probabilistic effect of the antigens' valence on the apparent affinity constant for antibodies coupled to agarose while the ascending limb of the Scatchard plots as compatible with cooperation between antibody active sites.

INTRODUCTION

Many cellular activities are initiated by the reaction of cell membrane bound receptors with specific ligands. The immune response is no exception: the production of antibody, the proliferation of immunocompetent cells, the activation of T-lymphocytes to reject tumors and transplants of foreign tissue, and some states of hypersensitivity are perhaps triggered by the reaction of antigen with cell surface receptors (Feldman & Globerston, 1976; Warner, 1974). Although some attempts have been made to closely examine the reaction of cell bound receptors with antigen (Davie *et al.*, 1973; Karush & Hornick, 1969), the low concentrations of specific cells and the restrictions imposed on the instrumentation by whole live cells limit the depth of such studies. In an effort to identify the most important factors in the binding of antigen by cell receptors, we have covalently coupled purified antibodies onto agarose beads and began a systematic exploration of the receptor–antigen reaction presumably occurring on the cells by way of this model system. In this paper special emphasis is made in describing the effects of the antigens' valence or number of identical binding sites per molecule on the binding process.

Our results certainly point to the importance of the number of dinitrophenyl (DNP) determinants carried by human serum albumin (HSA) on the apparent affinity (avidity) of its reaction with anti-DNP and anti-HSA antibodies fixed to a solid phase, and on the height of an ascending limb found in the Scatchard plots at low concentrations of bound ligand, compatible with cooperation between antibody sites.

* Abbreviations used: DNP, dinitrophenyl; HSA, human serum albumin; PBS, phosphate buffered saline.

MATERIALS AND METHODS

The overall experimental design consists of studying the reaction of anti-DNP or anti-HSA purified antibodies coupled onto agarose with tritium-labelled HSA dinitrophenylated to different extents (α) DNP$_\alpha$HSA-^{3}H).

Immunogens and immunization procedures

To obtain anti-DNP antibodies, DNP$_{23}$BSA was prepared by reacting 2 g of bovine serum albumin (BSA, Sigma Chem. Co.) with 350 mg of 1-chloro-2,4-dinitrobenzene (DNC1B, Sigma) in a 2% solution of K_2CO_3 at a pH of 9 and room temperature for 4 hr. Free DNC1B was eliminated by extensive dialysis against a phosphate–saline buffer, PBS (0.15 *M* NaCl, 0.01 *M* PO_4, pH 7.4). Protein concentration was determined by Nesslerisation (Campbell *et al.*, 1970) after total digestion of the conjugates with H_2SO_4 in a micro-Kjeldahl digestor. The DNP:protein molar ratio was calculated from the conjugates' O.D. at 363 nm using 17530 as the molar extinction coefficient for DNP-lysine (Eisen, 1964). Anti-HSA antibodies were obtained using 99% pure human serum albumin (Sigma) as immunogen. Two adult male sheep were immunized, one with HSA and the other with DNP$_{23}$BSA; both animals received 5 mg of antigen every 15 days intramuscularly using Freund's complete adjuvant with the antigen in only the first occasion. Sheep were bled one week after each immunization by puncture of the jugular vein obtaining 500 ml of blood each time. Sera were evaluated for antibody content by conventional agar double-diffusion precipitation methods (Campbell *et al.*, 1970). Precipitating sera were used as sources of purified specific antibodies.

Antibody purification

Anti-DNP and anti-HSA gammaglobulin fractions were

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REGULATION OF THE BINDING OF ANTIGEN TO RECEPTORS BY SOLUBLE ANTIBODIES: *IN-VITRO* COMPETITION AND SYNERGISM FOR DINITROPHENYLATED HUMAN SERUM ALBUMIN AND ε-DNP–LYSINE

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Abstract—Soluble anti-DNP antibodies competed or collaborated in the binding of dinitrophenylated human serum albumin to anti-DNP or anti-HSA antibodies attached to Sepharose depending, directly, on the ratio of the concentrations of soluble to insoluble antibodies and, inversely, on that of the antigen. Soluble antibodies competed or collaborated with the insoluble ones by polymerizing the antigen and either incorporating or not incorporating the insoluble antibodies into the polymer. Furthermore, a clear advantage of insoluble antibodies over soluble ones in the binding of antigen was found, and is perhaps indicative of the importance of the reactions' micro-environment and of the secondary non-specific forces that may be participating. Thus, by analogy, the role of circulating anti-hapten and anti-carrier antibodies on the regulation of the immune response may be either positive or negative depending on their concentration, on the number of cellular receptors available, on the micro-environment of the cell surface and on the magnitude of the antigenic challenge. A simple rule that relates some of these variables with the immune response is discussed.

INTRODUCTION

Of major concern in any theory where the occupation of a receptor by ligand acts as the trigger of a biological response, is how to explain the occurrence of ligand binding, particularly as the concentration of the reactants is usually extremely low and also because there is often abundant ligand degradation and non-specific-binding systems coexisting in the path of the ligands to the receptors. Compartmentalization and high affinity of the ligand–receptor reaction, as well as complex cellular interactions that favor the reaction or magnify the very few successful bindings of receptors, are usually proposed as the main factors participating in such a mechanism ever accomplishing its triggering function. The immune response seems to depend on such strategy (Feldmann & Globerston, 1976; Sercarz *et al.* 1974) but it presents a very special case in the multiplicity of receptors a ligand as complex as antigen can bind to. Indeed, receptors of a given specificity should not only compete for antigen with circulating antibodies of the same specificity, but also with receptors and antibodies directed against the other specificities carried by the antigen. Thus, the distribution of antigen on the total set of receptors and antibodies capable of binding it, is hard to predict and, therefore, the regularity of the immune response to a given antigenic determinant is a somewhat amazing event; unless, of course, the different parts of the binding system interact in some complex manner to direct the antigen towards a given subsystem. Circulating antibodies could indeed play a significant role in the regulation of the immune response (Uhr & Möller, 1968) via their influencing the distribution of antigen. Some hints on how receptor and antibody molecules could interact are already in the literature, mainly from studies on the effects of circulating antibodies on the immune response of whole animals or tissue cultures (Uhr & Möller, 1968; Graf & Uhr, 1969; Bystryn *et al.*, 1970; Haughton & Mäkelä, 1973; Diener & Feldmann, 1969; Abrahams *et al.*, 1973; Vann, 1969). Regulatory roles of soluble anti-hapten and anti-carrier antibodies are envisaged via precipitation of antigen, masking of antigenic determinants, bridging between interacting cells, and cross-linking of receptors, to name but a few. But due certainly to the experimental restrictions imposed by the condition of life, the hypotheses are in need of data that would rigorously test and quantitate the participation of each one of these mechanisms that are not mutally exclusive.

We set out to study the interactions between receptors, antibodies and antigen in determining the distribution of antigen in an *in-vitro* system, where receptors were modeled by anti-hapten and anti-carrier antibodies immobilized in agarose beads, circulating antibodies by soluble anti-hapten antibodies, and where the antigen was radioactive, dinitrophenylated human serum albumin (DNP_zHSA-^3H)* or dinitrophenyl–lysine (DNP–lysine-^3H). Our results indicate that soluble anti-

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* Dinitrophenyl will be abbreviated by DNP and human serum albumin by HSA. The subscript z in the antigens refers to the hapten:carrier molar ratio.

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Immunology

Crawling movements of lymphocytes on and beneath fibroblasts in culture

(activated lymphocytes/cell motility/T-cell leukemias/surface antigens)

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ABSTRACT Some lymphocytes become highly motile upon immunological stimulation *in vivo* or *in vitro*. When introduced into a culture of 3T3 or L cells and followed by live-cell microscopy, some of these lymphocytes were observed to crawl on top of, along the edges of, and preferentially beneath the attached fibroblasts. The crawling could be as rapid as 20 μm/min, easily detectable without a time-lapse device. The striking ability of crawling lymphocytes to penetrate beneath attached 3T3 cells provided a quantitative means to compare the crawling activity of different lymphocyte populations under various conditions. Crawling was diminished by inhibitors of energy metabolism, by agents that disrupt the cytoskeleton, and by absence of Mg^{2+} and Ca^{2+}, but not of Ca^{2+} alone. Crawling lymphocytes were virtually absent in normal thymus and spleen cells. They increased greatly in 5-day mixed lymphocyte cultures and in peritoneal exudate lymphocytes taken after mice had been immunized with allogeneic tumor cells. T cells accounted for most of the crawlers. Of two T-cell leukemias tested, RI^+ cells were crawlers whereas EL-4 cells were not. The *H-2* haplotype of the 3T3 fibroblasts (i.e., whether syngeneic or allogeneic) had no apparent effect on lymphocyte crawling activity. The crawling may relate to the exploration of cell surface antigens by lymphocytes (immune surveillance), to the mode of action of cytotoxic T cells, to the migration of lymphocytes across blood vessel walls, or to the penetration of lymphocytes into "solid" masses of normal tissue or tumor cells.

The interactions of lymphocytes with other cells are important in the induction and regulation of immune responses and in the expression of immune functions, such as the destruction of target cells bearing abnormal surface antigens. Very little is known about the ways in which lymphocytes explore the surface of other cells to hunt for appropriate surface antigens, or penetrate into a "solid" mass of cells, or cross endothelial or mesothelial boundaries to make contact with extravascular cells. The present paper describes an approach to the analysis of these exploratory and migratory activities. In particular, it describes the crawling behavior of lymphocytes on and beneath attached fibroblasts in culture and describes conditions for comparing the crawling activity of lymphocyte populations that have been activated in various ways.

MATERIALS AND METHODS

Mice and Cells. In the following list of mouse strains, tumors, and cell lines, the haplotypes of *H-2*, the major histocompatibility complex of the mouse, are given in parentheses. Mice of the BALB/c AnN (*d*) and of the congenic BALB.B (*b*), and BALB.K (*k*) strains were produced at the Massachusetts Institute of Technology. 3T3 fibroblasts derived from these mice were developed by Hale *et al.* (1). A line of L cells [transformed fibroblasts from the C3H/HeJ (*k*) strain] was obtained from H. Green. The T-cell lymphomas, RI^+ from C58/J (*k*) and EL-4 from C57BL/6 (*b*) mice, were obtained from M. Bevan and H. Winn, respectively. LPC-1, a myeloma of BALB/c AnN, was adapted to grow in culture (2). L cells, RI^+ and EL-4, and LPC-1 were grown in minimal essential medium (low Ca^{2+}), RPMI-1640, and L-15 (GIBCO), respectively, each supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Rockville, MD).

Lymphocyte Stimulation. Spleen cells were cleared of erythrocytes with NH_4Cl and resuspended in culture medium (1, 2). In mixed lymphocyte cultures, 7×10^6 "responder" spleen cells were incubated with 3×10^6 γ-irradiated (1000 rad; 1 rad = 1.00×10^{-2} J/kg) allogeneic "stimulator" cells in 2 ml of medium for 4–6 days (1, 2). Lymphocytes at 1.0×10^7 cells per ml were also stimulated with concanavalin A (Worthington) at 2 μg/ml or with lipopolysaccharides (Difco) at 10 μg/ml for 2 days to generate predominantly T- and B-cell blasts, respectively. BALB/c AnN mice were immunized by an initial ("priming") intraperitoneal injection of 2×10^7 EL-4 cells and a second ("booster") intraperitoneal injection 4 weeks later of 2×10^7 cells, either EL-4 cells or BALB.B spleen cells (both have the *H-2*^{b} haplotype).

Separation of B and T Cells. To obtain B lymphocytes, T cells were eliminated by treating spleen cells or cells recovered from mixed lymphocyte cultures or peritoneal exudates with anti-Thy-1 antibodies and complement (normal rabbit serum). The anti-Thy-1 was a 1:2000 dilution of ascites fluid from mice bearing a hybridoma that produces monoclonal anti-Thy 1.2 (3); dead cells were removed on a density gradient. To obtain T lymphocytes, the same mixed cell populations were passed through a nylon wool column to remove B cells and macrophages (4). Peritoneal exudate cells were incubated in a petri dish at 37°C for 1 hr to allow macrophages to adhere to the plate; only the nonadherent cells (lymphocytes) were tested.

Live-Cell Microscopy. Chambers for visualizing cells by microscopy were constructed essentially as described (5). L or 3T3 cells (2×10^5) in 5 ml of Dulbecco's modified Eagle's medium (GIBCO)/10% serum were plated on a 75 × 25 mm glass slide (Fisher) in a 100-mm petri dish (Falcon). After 4 hr (37°C in 10% CO_2/90% air) the cells were covered by a 22 × 22 mm glass cover slip (Corning) supported by bits of cover slip (0.1-mm thick) at each corner to form a chamber. Lymphocytes at 1.0×10^6 cells/ml in the same medium were introduced into the chamber, which was then sealed along its edge with nontoxic wax. While the chamber was observed under a Zeiss microscope (Model 2432, equipped with a camera), it was maintained at 37°C with an Air Curtain incubator (Sage, Cambridge, MA). Cells remained viable and active for more than 12 hr, but observations were generally completed in 3–4 hr. Photographs were taken with Kodak PX panchromatic film (Eastman Kodak) at ×160 or ×400 magnifications.

Assay for Lymphocytes That Crawl underneath 3T3 Cells. 3T3 cells (1.5×10^5) in 5 ml of medium were plated on a 60-mm petri dish, and after 4 hr 2.0×10^6 lymphocytes, whose crawling

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CYCLICAL CHANGES IN SUSCEPTIBILITY OF A MYELOMA TUMOR (LPC-1) TO IMMUNE DESTRUCTION

I. Changes in Reactivity with Cytotoxic T Lymphocytes and Anti-H-2^d Sera[1]

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One of six transplantable ascites tumors of BALB/c mice was found to become periodically resistant to cytotoxic T lymphocytes (CTL). About 12 days after LPC-1, a myeloma tumor, was transplanted it became resistant to lysis by allogenic CTL (anti-H-2^d) and by CTL directed to trinitrophenyl groups or minor histocompatibility antigens. Susceptibility to lysis by all of these CTL was regained within 2 to 4 days after transfer of the resistant cells to a fresh BALB/c host. These changes were recurrent: in each transplantation cycle the early LPC-1 cells were susceptible and the late cells were resistant to CTL. Analyses with antisera (B10 anti-B10.D2) showed that the serologically recognized products of the H-2^d haplotype were reduced about 10-fold on the LPC-1 cells that were resistant to CTL.

Lysis of tumor cells by cytotoxic thymus-derived lymphocytes (CTL)[6] probably constitutes the major host immune defense against many proliferating tumors. In general, the specificity of CTL is restricted by products of the major histocompatibility complex (MHC): i.e., for lysis to occur, the target cell must present on its surface both the antigen and the MHC products that were present on the stimulator cells that originally elicited the development of the CTL (1).

Besides surface antigens and MHC products, additional properties of the target cell surface are probably necessary for lysis by attacking CTL. This possibility is in accord with previous studies where it was shown that when MOPC-315EL, a BALB/c myeloma tumor, proliferates *in vivo* in BALB/c mice as a transplanted ascites tumor the tumor cells undergo late in each transplantation cycle a loss in ability to react with CTL, followed by prompt restoration of reactivity when the "late" tumor cells are transplanted into a fresh host (2). This reversible change, recurrent in each transplantation cycle, was evident with CTL having two kinds of activities. First, where CTL and tumor cells differed in MHC (H-2) haplotype and the CTL were directed against H-2 products on the tumor target cells; second, where the CTL and tumor cells had the same H-2 haplotype and the CTL were directed to non-H-2 antigens (trinitrophenyl groups or minor histocompatibility antigens) (3–5). Although the late MOPC-315EL cells were defective as targets in a variety of assays for CTL activities, the serologically defined H-2 products (H-2^d) were no different, qualitatively or quantitatively, on the late, CTL-resistant and the early, CTL-sensitive tumor cells (2). Hence, other target cell properties, in addition to surface antigens and H-2 products, are probably necessary for lysis by CTL.

The present study was undertaken initially to determine how frequently periodic resistance to CTL occurs among other transplanted tumors of BALB/c mice. We show here that of six tumors examined (five myeloma tumors and Meth A, a fibrosarcoma) one myeloma tumor, LPC-1, also undergoes late in its transplantation cycle through BALB/c mice a loss in reactivity with various CTL (anti-H-2^d, anti-Tnp, anti-minor histocompatibility antigens), and recovery of reactivity upon transfer into fresh BALB/c hosts. However, analysis with anti-H-2^d antisera showed that, unlike MOPC-315EL, a periodic loss of the serologically recognized H-2^d products also occurred with LPC-1 and coincided roughly in time with this tumor's loss of reactivity with CTL.

MATERIALS AND METHODS

Animals. BALB/c AnN and congenic BALB.K mice (H-2 haplotypes are H-2^d and H-2^k, respectively) were produced at the Massachusetts Institute of Technology from breeders pro-

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[6] Abbreviations used in this paper: CTL, cytotoxic thymus-derived lymphocytes; MHC, major histocompatibility complex; Tnp, 2,4,6-trinitrophenyl; PBS, phosphate buffered saline (0.15 M NaCl-0.01 M K phosphate, pH 7.4); TNBS, 2,4,6-trinitrobenzenesulfonic acid; C, complement; K medium, RPMI 1640 supplemented as described (2).

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CYCLICAL CHANGES IN SUSCEPTIBILITY OF A MYELOMA TUMOR (LPC-1) TO IMMUNE DESTRUCTION

II. Periodic Fluctuations During Growth in Normal and Nude Mice and in Culture[1]

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Cells of the LPC-1 myeloma tumor (of BALB/c origin) were tested periodically for susceptibility to lysis by anti-H-2 cytotoxic T lymphocytes (CTL) and by complement-dependent anti-H-2 antibodies (Ab), and for ability to adsorb these Ab, as the cells grew in culture and in ascites form in nude (BALB/c•nu) and in normal BALB/c mice. Cells harvested from 2 to 8 days after inoculation into the peritoneal cavity had the "early" phenotype: they were extensively lysed by the CTL and Ab and effectively adsorbed the Ab. Cells harvested on day 8 and afterward had the "late" phenotype: they were not lysed by CTL, required more Ab for lysis, and had about 8-fold less ability (per cell) to adsorb the Ab. All of these activities were completely reversed 2 to 4 days after transferring late cells into fresh BALB/c hosts. LPC-1 cells growing in nude BALB/c mice also changed from the early, CTL-susceptible to the late CTL-resistant phenotype. With repeated cell counts and measurements of ^{125}I-iododeoxiuridine incorporation to estimate growth, it was found that exponentially growing cells had the early phenotype and that cells in stationary phase had the late phenotype. When placed in culture, the early cells retained their phenotype indefinitely (up to 40 days). However, the late cells switched phenotype: initially resistant to CTL and Ab, after 1 to 4 days in culture they became fully susceptible to CTL and highly reactive with the Ab, and these features persisted in culture thereafter. This transition occurred even when the late, resistant cells were prevented from dividing in culture by prior irradiation (10,000 R) or by trinitrophenylation with 2,4,6-trinitrobenzenesulfonic acid. The findings in both nude mice and in culture indicate that the reversible, early-late phenotypic changes are due to changes in individual cells, rather than to immunoselection of preexisting variant cells.

The accompanying paper describes changes in immunologic reactivity of a BALB/c plasmacytoma, LPC-1, during growth as an ascites tumor in syngeneic BALB/c hosts (1). Four days after transfer to a BALB/c mouse the harvested tumor cells were readily lysed by cytotoxic thymus-derived lymphocytes (CTL)[6] and by complement- (C) dependent cytotoxic antisera directed to products of the major histocompatibility complex (anti-H-2^d CTL and antibodies). However, 10 to 14 days later the harvested tumor cells were highly resistant to these CTL and somewhat resistant to the antisera. The resistance also extended to CTL that had the same H-2 haplotype as the tumor and were directed against either trinitrophenyl (Tnp) groups (2) or minor histocompatibility antigens (minor H) (3) on the LPC-1 target cells. On being transferred to a fresh BALB/c host the resistant LPC-1 cells regained their susceptibility to lysis by the CTL and antiserum (1).

To explore these periodic changes further, we have here characterized them more closely during growth of LPC1 cells in both normal and nude mice and in culture. The results show that the changes in reactivity with CTL and anti-H-2^d antibodies coincide in time, that the changes occur cyclically during growth in nude as well as in normal BALB/c mice, and that some of the changes also occur when LPC-1 cells are maintained in culture, even when cell division is blocked by γ-irradiation or by trinitrophenylation with 2,4,6-trinitrobenzenesulfonic acid.

MATERIALS AND METHODS

Mice and cytotoxicity assays. The C57BL10(B10), B10.D2,

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[6] Abbreviations used in this paper: CTL, cytotoxic thymus-derived lymphocytes; Tnp, 2,4,6-trinitrophenyl; IUdR, iododeoxyuridine; PBS, phosphate-buffered saline (0.15 M NaCl-0.01M K phosphate, pH 7.4); TNBS, 2,4,6-trinitrobenzenesulfonic acid; minor H, minor histocompatibility antigens; C, complement.

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CYCLICAL CHANGES IN SUSCEPTIBILITY OF A MYELOMA TUMOR (LPC-1) TO IMMUNE DESTRUCTION

III. Periodic Production of a Cell Surface Glycoprotein and Changes in Reactivity with Cytotoxic T Cells and Anti-H-2^d Sera[1]

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Previous studies showed that when LPC-1 myeloma cells were grown as ascites tumor cells in syngeneic (BALB/c) mice, the cells harvested after 2 to 4 days of growth ("early" cells) were susceptible to lysis by cytotoxic T lymphocytes (CTL) and highly reactive with anti-H-2^d antisera. Cells harvested after 12 to 14 days of growth ("late" cells) were resistant to lysis by CTL and poorly reactive with anti-H-2^d antisera. The present study shows that exposure to trypsin or chymotrypsin or subtilisin (but not to thrombin or Staphylococcus A protease) promptly converts LPC-1 cells with the late phenotype into cells with the early phenotype. Comparison of radiolabeled cell surface proteins by gel electrophoresis showed that the late cells possess a prominent trypsin-sensitive, high m.w. (160,000 daltons) surface glycoprotein that is present in smaller amounts on the early LPC-1 cells. This glycoprotein (gp160) was not detectable in four other BALB/c tumors that do not undergo the early-late transition of LPC-1. That gp160 was produced by LPC-1 cells, rather than adsorbed by these cells as they grow *in vivo*, was evident from the presence of an indistinguishable metabolically labeled glycoprotein on cultured LPC-1 cells.

When the transplantable plasmacytoma LPC-1 is grown as an ascites tumor in syngeneic BALB/c mice, the tumor cells undergo cyclical changes in susceptibility to lysis by cytotoxic thymus-derived lymphocytes (CTL)[4] (1, 2). During the first week of a 2- to 3-week transplantation cycle, the LPC-1 cells are readily lysed by allogeneic (anti-H-2^d) CTL or by syngeneic CTL with H-2-restricted specificity for non-H-2 antigens (Ag), either naturally occurring minor histocompatibility Ag or artificially introduced trinitrophenyl (Tnp) groups on these cells. Beginning with about day 10, however, the LPC-1 cells become progressively more resistant to all of these CTL. The late, resistant cells again become susceptible to lysis when they are a) transferred to a fresh BALB/c host, and harvested within a week, or b) maintained in culture for more than 4 days (1, 2). As the LPC-1 cells grow *in vivo* and lose susceptibility to lysis by CTL, they also become less reactive with antibodies (Ab) to products of the H-2 complex: the late cells are less readily lysed than early cells by anti-H-2^d Ab and complement (C) and they become 6 to 10 times less effective, per cell, than the early cells in adsorbing these Ab (1, 2). All of these changes in the LPC-1 cells, as they progress through the growth cycle, can be explained by either a decrease in synthesis of H-2-specified surface glycoproteins, or a progressive decrease in the surface display of these Ag due, for instance, to increasing production of other surface macromolecules that cover or mask the H-2 Ag.

In the present study we show that exposure to trypsin and certain other proteases promptly converts LPC-1 cells with the "late phenotype" (resistant to CTL and poorly reactive with anti-H-2 Ab) into cells with the "early phenotype" (susceptible to CTL and highly reactive with anti-H-2 Ab). In addition, comparison of radiolabeled cell surface glycoproteins by polyacrylamide gel electrophoresis revealed that the late, resistant cells possess a prominent trypsin-sensitive, high m.w. surface glycoprotein that is present in only small amounts on the early LPC-1 cells. This glycoprotein ("gp160") was not detectable in four other BALB/c tumors that do not undergo the early-late transition of LPC-1. All of these observations suggest that as LPC-1 cells cease to proliferate near the end of each transplantation cycle, they produce a glycoprotein that accumulates on the cell surface, masks H-2 surface Ag, and renders the cells resistant to host immune defenses (CTL and Ab plus C); on resuming growth in a fresh host, the surface glycoprotein is reduced and the cells regain susceptibility to immune lysis until

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[4] Abbreviations used in this paper: CTL, cytotoxic thymus-derived lymphocytes; minors, minor histocompatibility antigen. PBS, phosphate-buffered saline (0.15 M NaCl-0.01 M K phosphate, pH 7.4), SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

INTERACTIONS BETWEEN A NOVEL CELL SURFACE GLYCOPROTEIN AND THE H-2K AND H-2D ANTIGENS ON MYELOMA TUMOR CELLS*

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Abbreviations used in this paper: Ags, antigens; Abs, antibodies; CTL, cytotoxic T lymphocytes; PBS, phosphate buffered saline (0.15M NaCl-0.01M K phosphate, pH 7.4); MHC, major histocompatibility gene complex.

SUMMARY

Previous studies showed that when a subline of plasmacytoma LPC-1 is grown in ascites form the tumor cells harvested after 10-12 days ("late" cells) are resistant to lysis by cytotoxic T lymphocytes (CTL), apparently because the cell surface H-2 encoded molecules are blocked by a trypsin-sensitive glycoprotein of approximately 160,000 daltons: the glycoprotein (gp160) is produced, and accumulates on the cell surface, as the growing cells enter stationary phase. In contrast, LPC-1 cells harvested from the peritoneal cavity early in the transplantation cycle (ca 4 days), while in exponential growth ("early" cells), have far less gp160 and are susceptible to lysis by CTL. We show here that, compared with early cells, the late LPC-1 cells are far less effective in adsorbing antibodies (Abs) to products of the H-2K^{d} and H-2D^{d} alleles, but only slightly less effective in adsorbing Abs to Ia antigen, and fully effective in adsorbing Abs to several antigens not related to H-2 (e.g., PC.1, gp70, gp100, B220). The differences suggest that gp160 might preferentially block H-2 encoded molecules (especially K and D).

Gp 160 was also detected on another myeloma, MOPC-315-OPEC, a subline of MOPC-315, where it also appeared to block H-2 molecules but not PC.1. Additional evidence for an interaction between gp160 and H-2 molecules was obtained when sonicated membrane fragments were used to elicit secondary CTL responses to H-2 in culture: the response could be elicited by fragments from early LPC-1 cells but not from late LPC-1 cells, unless the late cells were treated with subtilisin (to remove gp160) before sonication.

1. INTRODUCTION

The cell surface glycoproteins encoded by the genes of the major histocompatibility gene complex (MHC) exert a major influence on a wide variety of cellular immune reactions (1-4). Other cell surface molecules that interact with the products of the MHC are thus of potential interest. The observations described in this paper suggest that they may be a selective interaction between the cell surface molecules encoded by the H-2K and H-2D loci and another cell surface glycoprotein (gp160) that is expressed on some myeloma cells.

The glycoprotein called gp160 was identified in previous studies on the resistance of myeloma tumors to lysis by cytotoxic T lymphocytes (CTLs). When cells of one of the resistant tumors, LPC-1, were grown in ascites form and harvested after 4 days ("early cells") they were readily lysed by CTLs that were specific for either H-2 or non-H-2 antigens (Ags) on these cells [e.g., trinitrophenyl (Tnp) or minor histocompatibility Ags ("minors")]. With continued growth, however, the cells became progressively less reactive, and when harvested on day 12 ("late cells") they were completely resistant to lysis by CTLs (anti-H-2, anti-Tnp, anti-minors). The capacity to adsorb anti-H-2 antibodies (Abs) changed in parallel: the late cells were 6-10 times less effective than early LPC-1 cells in adsorbing these Abs. The late-resistant LPC-1 cells again became reactive with CTLs and anti-H-2 Abs 4 days after they were transferred into new hosts or placed in tissue culture (5,6).

The development of resistance by the late cells was correlated with the accumulation, on the cell surface, of a trypsin-sensitive glycoprotein of approximately 160,000 daltons (gp160) (7). Thus treatment of the late LPC-1 cells with trypsin, chymotrypsin, or subtilisin (but not with certain other proteases) promptly rendered them as reactive with CTL and anti-H-2 Abs as the early LPC-1 cells. The key glycoprotein was composed of a single polypeptide chain, and was synthesized by the LPC-1 cells (i.e., it was not adsorbed from the serum or ascites fluid of the host). Overall, these findings suggested that when gp160 is present in large amounts on the surface of late LPC-1 cells it blocks access of both CTL and Abs to the cell's H-2 Ags.

Prompted by these observations we have now asked whether the presence of gp160 also blocks access of Abs to other, non-H-2, Ags on the surface of the late LPC-1 cells. The results show that, unlike the H-2 Ags, the four non-H-2 surface Ags tested appear to be just as accessible on the late LPC-1 cells as on the early cells, suggesting that gp160 might interact preferentially with H-2 encoded molecules on the cell surface. Another myeloma tumor that is reported here to also express gp160 on its surface (MOPC-315-OPEC), also behaves in adsorption tests with anti-H-2 and anti-PC.1 Abs as though gp160 interacts selectively with the H-2 encoded molecules. However, when measured by immunoprecipitation and electrophoresis, the amounts (per cell) of the H-2 Ags and a non-H-2 Ag (PC.1) varied in diverse cells in such a way as to suggest that differences in Ab-adsorbing ability of various cells could reflect differences in either amount or in accessibility of surface Ags. The results also show that the interaction between gp160 and H-2 seems to be preserved in sonicated membrane fragments from the late LPC-1 cells: the fragments from early LPC-1 cells were able to elicit a specific

secondary anti-H-2 CTL response in vitro, whereas the corresponding membrane fragments from the late LPC-1 cells were unable to do so, unless gp160 was removed from the late cells, prior to sonication, by treatment with subtilisin.

2. MATERIALS AND METHODS

2.1 Mice. BALB/cAnN (H-2^d), BALB.K (H-2^k), B10.D2 (H-2^d) and DBA/2 (H-2^d) mice were produced at the Massachusetts Institute of Technology. NZB (H-2^d) mice were purchased from the Jackson Laboratories, Bar Harbor, ME. Animals of the same sex and age (6-10 weeks) were used in each experiment.

2.2 Tumors. BALB/c plasmacytomas LPC-1, MOPC 315 and MOPC-167, originally obtained from Michael Potter, National Cancer Institute, were transferred each week as $1x10^7$ ascites tumor cells (per BALB/c AnN mouse). Early and late LPC-1 cells were obtained after 4 and 12-14 days of growth, respectively, by flushing the peritoneal cavity with phosphate buffered saline (PBS); red blood cells were eliminated by several cycles of centrifugation at 50 x G for 7 min in PBS. The DBA/2 tumor P815 was maintained in culture. Cells were labeled with ^{51}Cr by incubating $5x10^6$ cells with 250 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear) in 0.5 ml of RPMI-1640, with 10% fetal calf serum, for 1.5 hrs at 37^o.

2.3 Sera. The following antisera to products of the H-2^d complex were used: 1) B10 anti-B10.D2, directed against the entire H-2 complex; 2) (B10xA) anti-B10.D2 (anti-H-$2K^d$ and presumably specific for H-2.31); and 3) (B10.AKMx129) anti-B10.A (anti-H-$2D^d$, and presumably specific for H-2.4). These antisera were produced in this laboratory by biweekly intraperitoneal injections of $1x10^7$ spleen cells. The specificity of these antisera was tested in two ways:

1) In cytotoxic assays, using cells of the H-2^d, H-2^b and H-2^K haplotypes. The anti-H-$2K^d$ and anti-H-$2D^d$ sera were found to lyse only targets of the H-2^d haplotype. The antisera directed to the entire H-2^d region (B10 anti-B10.D2) lysed the H-2^d cells and cross reacted as expected with the H-2^K cells. 2) Immunoprecipitations of NP-40 extracts of surface labeled cells with ^{125}I (see below) showed that all three antisera precipitated a protein of approximately 45,000 daltons and that two of the antisera (B10 anti-B10.D2 and anti H-$2K^d$) also precipitated a peak of 70,000 daltons, presumably gp70. The anti-gp70 Abs were irrelevant for the present studies because they had no cytotoxic activity on gp70-positive cells with different H-2 haplotype. An anti-Ia serum (A.TH anti-A.TL serum) was kindly provided by Dr. David Sachs, National Cancer Institute. This serum was found to immunoprecipitate from ^{125}I-labeled LPC-1 cells a labeled protein that migrated in SDS-PAGE as a broad peak of approximately 30,000 daltons.

NZB, DBA/2, and B10.D2 mice were injected intraperitoneally on day 0 with $1x10^7$ viable LPC-1 cells. The tumor grew at first but was usually rejected by day 21; the animals were boosted with $1x10^7$ viable LPC-1 cells on day 28 and every 15 days thereafter, and were bled 7 days after each injection. Serum from the B10.D2 mice were adsorbed 8 times, each time with an equal volume of MOPC 167 cells, for 30 min at 4^o; after the adsorptions these sera had no cytotoxicity activity against ^{51}Cr-labeled MOPC-167 cells but were still active against LPC-1 cells.

Goat anti-tween-80-solubilized Maloney virus was kindly provided by Dr. David Baltimore. Rabbit anti-mouse thymocyte serum was purchased from Cappel Labs, Cochranville, PA.

2.4 Immunoprecipitation and Analysis of Radiolabeled Surface Proteins. LPC-1, MOPC-315-OPEC and MOPC-167 cells were labeled with $Na^{125}I$ (New England Nuclear) by the lactoperoxidase method (7). To solubilize the membrane proteins the radiolabeled cells were incubated for 30 min on ice with 0.5% NP-40 in PBS (200 µl per $1x10^7$ cells); debris was removed by centrifugation at 8000 x G for 20 min. 100 µl of the NP-40 extract were mixed with 20 µl of antiserum and, after 15 min incubation at 25°, 100 µl of suspension of formaldehyde-fixed and heat-killed stapylococcus A cowan strain I (8) (10% suspension in PBS-NP-40) were added. After an additional incubation for 15 minutes, the bacteria were packed and washed 5 times, each time by centrifugation (300 x G, 10 min) in PBS-NP-40. The bound radiolabeled components were then eluted by incubating the bacteria in 50 µl of 5% SDS in 0.1M tris, pH 6.8, 4% ß-mercaptoethanol in a boiling water bath for 2 min. The samples were centrifuged at 8000 x G for 10 min to remove the staph A and the supernatant was subjected to SDS-polyacrylamide gel electrophoresis in tube gels (SDS-PAGE) as described (9). Gels were frozen and sliced on dry ice (1 or 2 mm per slice), and the radioactivity of each slice was measured in a Packard gamma counter.

2.5 Antibody Adsorption Tests. Quantitative adsorption tests were performed by incubating different numbers of tumor cells on ice for 30 min with 75 µl of diluted antiserum. (The dilution used gave, in separate tests with complement (C), approximately 60% lysis of the appropriate ^{51}Cr-labeled tumor cells). The cells, antiserum, and C were all diluted in RPMI-1640 containing 0.02M HEPES buffer and 0.1% Gelatin (Difco). The adsorbing cells were removed by centrifugation and two aliquots of the adsorbed antiserum, 25 µl each, were placed in separate wells of a 96-well microtiter plate (Limbro) and tested for residual cytotoxic activity by adding $5x10^4$ ^{51}Cr-labeled cells in 25 µl containing normal rabbit serum, diluted 1:10, as a source of C. Afte

incubation at 37° for one hr, 100 µl of ice-cold medium was added to each well and the plates were centrifuged at 100 x G for 10 min; radioactivity in 75 µl of the supernatant from each well was measured in a Packard gamma counter. Specific lysis was calculated as 100 (ER-SR/100-SR), where ER (experimental release) is the % ^{51}Cr released in the presence of serum and C, and SR (spontaneous release) is the % ^{51}Cr released in the presence of C alone.

2.6 Stimulation of Secondary CTL Responses. $7x10^6$ spleen cells from BALB.K mice, primed 4 to 6 weeks previously by an intraperitoneal injection of $1x10^7$ BALB/c spleen cells, were incubated with either $1.7x10^5$ irradiated (10,000 Rads) LPC-1 cells or different amounts of membranes obtained by sonicating LPC-1 cells (see below). After incubating the cultures in supplemented RPMI-1640 medium (7) at 37° in a 10% CO_2-90% air mixture for 5 days, the remaining cells (effectors) were tested for cytotoxic activity against ^{51}Cr-labeled H-2^d target cells (P815, below). Crude membranes from LPC-1 cells were obtained under sterile conditions as previously described (10-12) by first sonicating $1x10^8$ cells in 10 ml of PBS, on ice (six times, 30 sec each time). Whole cells and debris were removed by centrifugation at 100 x G for 10 min. The supernatants were centrifuged at 200,000 x G for 60 min and the pelleted membranes were resuspended in 1 ml of RPMI-1640 without serum. Protein content was determined by the Lowry method (13).

2.7 CTL-Mediated Cytotoxicity Assays. Different numbers of effector cells were incubated with $1x10^4$ ^{51}Cr-labeled P815 target cells in a final volume of 200 µl of supplemented RPMI-1640. After 4 hrs at 37° under 10% CO_2, 1.2 ml of ice-cold PBS was added to each tube and the cells were centrifuged (100xG, 5 min). Cytotoxicity was measured by determining the radioactivity released

into the supernatant. Specific lysis was calculated as 100 (ER-SR/100-SR), where ER (experimental release) and SR (spontaneous release) are percentages of ^{51}Cr released from target cells in the presence and absence of immune cells respectively.

3. RESULTS

To determine whether gp160 has different effects on cell surface H-2 and non-H-2 Ags we compared the adsorption of Abs to these various cell surface Ags by myeloma tumor cells that differ in amounts of surface gp160: 1) early vs. late LPC-1 cells, 2) late LPC-1 and MOPC-315-OPEC vs. MOPC-167.

The non-H-2 Ags examined were identified by the following antisera:

1) DBA/2 anti-LPC-1. Figure 1A shows the SDS -PAGE pattern of the ^{125}I-labeled Ag bound by this antiserum in the NP-40 extract of ^{125}I-labeled LPC-1 cells. A single peak of approximately 110,000 daltons was evident. The antiserum and the position of this peak indicate that the Ag corresponds to the plasma cell Ag, PC.1, described by Takagashi et al. (14) and Tung et al. (15).

2) B10.D2 anti-LPC-1. The SDS-PAGE profile, shown in Figure 1B, indicates that this antiserum contained both Abs to PC.1 and Abs to some Ags of lower molecular weight. After exhaustive adsorption with MOPC-167 cells, most of the anti-PC.1 activity was removed, leaving Abs to the other Ags (Fig. 1B).

3) Rabbit anti-mouse thymocyte serum. This antiserum, obtained from a commercial source, has been shown to react with a molecule of 200,000 daltons on the surface of murine thymocytes (T-200), and with a similar antigen of 220,000 daltons on B cells (16). Figure 1C shows that this antiserum binds

a cell surface ^{125}I-labeled component of high molecular weight in NP-40 extracts of LPC-1 cells. We tentatively designate this Ag as B-220.

4) Goat anti-Maloney virus. This antiserum bound two proteins, approximately 70,000 and 100,000 daltons, from LPC-1 cells (Fig. 1D). This is in accord with the known expression of leukemia virus antigens (gp70, gp100) on many mouse myeloma cells (17).

3.1 Accessibility of H-2K, H-2D, and Ia Antigens on Intact LPC-1 Cells. We previously reported that late LPC-1 cells adsorbed 6-10 times less anti-H-2 Abs than early LPC-1 cells (5,6). The anti-H-2 Abs tested previously were raised against the entire H-2^d region by immunizing B10 mice with spleen cells from B10D2 mice. It is shown in Figure 2, with antisera having more restricted specificities, that Abs against H-2K^d and Abs against H-2D^d Ags were also adsorbed markedly less (10-15 fold) by the late LPC-1 cells than by the early ones. There appeared also to be a small difference (about two-fold) in the adsorption of anti-Ia Abs by late and early LPC-1 cells, but whether this difference, observed in two different experiments, is significant, is not yet clea

3.2 Accessibility of the Non-H-2 Antigens on Intact LPC-1 Cells. Figure 3 shows that intact late and early cells did not differ significantly in their capacity to adsorb cytotoxic activity from the antisera to any of the four non-H-2 Ags tested.

3.3 Comparison of Three Myeloma Tumors. To determine whether the preferential blocking of the H-2 Ags by gp160 could be observed in additional tumors. we compared two other myelomas with LPC-1. One of them, a subline of MOPC-315 (called MOPC-315-OPEC) evidently expresses gp160 on its cell surface, whereas the other one, MOPC-167, does not. Thus, as shown in Figure 4, when the

^{125}I-labeled surface components were analysed by SDS-PAGE, both LPC-1 and MOPC-315-OPEC contained a prominent peak of approximately 160,000 daltons (arrows); this peak apparently was absent on MOPC-167 cells (panels A,B, and C). When Abs from NZB mice immunized with LPC-1 cells (see Materials and Methods) were used to immunoprecipitate the NP-40 solubilized, ^{125}I-labeled surface components from these three myelomas, gp160 was precipitated (along with a few other Ags) from both LPC-1 and MOPC-315-OPEC (Fig. 4, panels D and E) but not from MOPC-167 (panel F).

In Figure 5, the ability of LPC-1, MOPC-315-OPEC, and MOPC-167 cells to adsorb anti-H-2 and anti-PC.1 Abs is compared. These results show that to reduce the cytotoxic titer of the anti-H-2 Abs (B10 anti-B10.D2), 10 times more MOPC-315-OPEC and 50 times more LPC-1 cells were needed than MOPC-167 cells (panel A). In contrast, the three myeloma tumor cells were equally effective in adsorbing the Abs to PC.1 (DBA/2 anti-LPC-1, panel B).

3.4 Quantity of H-2 Encoded Antigens on Three Myeloma Tumors. To determine if the H-2 Ags were present on the surface of LPC-1 and MOPC-315-OPEC cells, but simply not accessible to Abs, the amounts of H-2 in NP-40 extracts of the myeloma cells were measured. The cells were surface-labeled (^{125}I and lactoperoxidase), extracted with NP-40, after precipitating the solubilized H-2 Ags with anti-H-2^{d} antisera B10 anti-B10.D2), the precipitates were subjected to SDS-PAGE. Table I shows that the total number of cpm migrating in the region of the H-2K and H-2D encoded heavy chains (45,000 dalton) was approximately the same in tumors that have gp160 (LPC-1 and MOPC-315-OPEC) and adsorb anti-H-2 Abs poorly and in the one that lacks gp160 and absorbs anti-H-2 Abs well. Also, the relative amount of H-2 of these cells (cpm H-2/to cpm) was approximately the same in MOPC-167 and MOPC-315-OPEC; in LPC-1 cells (Table I) the relative amount was somewhat less (Table 1).

A preliminary similar experiment with anti-PC.1 Abs suggested that late LPC-1 cells may have two times more PC.1 than early LPC-1 cells and four times more than MOPC-167 cells (data not shown).

3.5 Stimulation of Secondary CTL Response to H-2 Antigens by Subcellular Fragments of LPC-1 Cells. It is known that cell membrane fragments can elicit a secondary anti-H-2 CTL response in cultures of spleen cells from mice that wer primed by a previous injection of the corresponding intact allogeneic cells (10-12). Accordingly, to determine whether the apparent selective interaction between gp160 and H-2K and H-2D Ags occurs only on intact cells, we compared fragmented membranes from early and late LPC-1 cells for their ability to elicit secondary anti-H-2^d CTLs. Spleen cells from BALB.K mice that were primed 30 days previously with $1x10^7$ BALB/c spleen cells were stimulated with intact early or late LPC-1 cells and with different amounts of sonicated membranes from these cells.

As is shown in Table II, intact late LPC-1 cells were as effective as intact early cells in stimulating anti-H-2^d CTL responses by primed BALB.K spleen cells. This result is not surprising since, as we reported earlier (6), late LPC-1 cells revert to the early phenotype when placed in culture. However, crude membrane fragments from the late LPC-1 cells were distinctly less effective than membrane fragments from the early cells in eliciting anti-H-2^d CTLs. But, when the late LPC-1 cells were treated with subtilisin (to remove gp160) and then sonicated the resulting membrane fragments were just as effective as those from the early cells, suggesting that in sonicated

membrane fragments from late LPC-1 cells, the H-2K and D Ags are blocked by gp160.

4. DISCUSSION

The results of the present study indicate that Abs to H-2K and H-2D Ags are bound much less by late than by early LPC-1 cells (Fig. 2). In contrast, there is no difference between the early and late cells in ability to bind Abs to four non-H-2 Ags on these cells (Fig. 3). Additional evidence that suggests preferential blocking of the H-2 Ags by gp160 was obtained when three different myelomas, two that express gp160 on their surface (LPC-1 and MOPC-315-OPEC) and one that does not (MOPC-167, Fig. 4) were compared for their capacity to react with anti-H-2 and anti-PC.1 Abs. While these cells differed greatly in capacity to adsorb the anti-H-2^d Abs (Fig. 5A), no difference was observed with the anti-PC.1 Abs (Fig. 5B). Taken together, all of these results indicate that on the surface of late LPC-1 cells gp160 interacts selectively with Ags encoded by the H-2K and H-2D loci.

An alternative possibility is that late LPC-1 cells make much less H-2K and H-2D Ags than early LPC-1 or other myeloma cells. However, earlier studies showed that protease treatment of late LPC-1 cells removed gp160 and increased the cells' ability to react with anti-H-2 Abs (and CTLs), indicating that in the late LPC-1 cells the H-2 Ags are inaccessible, not absent (7). To evaluate this issue further, advantage was taken of the fact that detergent solubilization of plasma membranes can uncover antigenic determinants (epitope of membrane molecules that are not accessible to Abs on the intact cell (20); hence quantitative immunoprecipitation can be used to measure the amounts of labeled surface Ag on cells. When late and early LPC-1, MOPC-315-OPEC

and MOPC-167 cells were ^{125}I-surface labeled and solubilized with NP-40, approximately the same amount of surface, radioiodinatable H-2 Ag were detected on all of them (Table I). Thus, the H-2 Ags are not reduced in amount on the surface of LPC-1 and MOPC-315-OPEC; instead, they are blocked on the intact cells.

The same quantitative issue must be raised for the other, non-H-2 Ags. A preliminary measurement by immunoprecipitation with anti-PC.1 Abs, suggests that the late LPC-1 cells have approximately two times more PC.1 than the early LPC-1 cells and four times more than MOPC-167. If these results are substantiated, then the ability of late LPC-1 cells to adsorb anti-PC.1 Abs only as well as early LPC-1 and MOPC-167 cells would mean that PC.1 is somewhat less accessible on the surface of cells that have large amounts of gp160 (late LPC-1) than on gp160-negative cells (MOPC-167). Nonetheless, the blocking effect of gp160 seems to be clearly more pronounced on H-2K and H-2D Ags than on the other cell surface Ags tested.

From the capacity of fragmented membranes from sonicated early, but not from sonicated late, LPC-1 cells to elicit secondary anti-H-2 CTL in culture, it is likely that gp160 blocks H-2K and H-2D Ags on isolated membrane fragments from the late cells (Table II). Thus, there is probably no need for complex structures in the intact cell (such as cytoskeleton) to maintain the gp160-H-2K,D interaction.

In results that are roughly similar to ours,Tokuyama and Migita (18) reported that a trypsin-sensitive glycoprotein of 120-160,000 daltons, present on the BALB/c myeloma tumor P58-8, blocks access of Abs to H-2 Ags on these tum cells. It is possible that gp160 and the blocking glycoprotein on P58-8 cells are the same; however, the same group reported earlier that some undefined

non-H-2 Ags recognized by a rabbit anti-P58-8 serum (adsorbed to remove anti-H-2 Abs) were also blocked on P58-8 cells with the surface glycoprotein (19).

Different approaches have been followed to study the interaction of the H-2 Ags with various cell surface molecules. Some Ags of viral origin seem to migrate with H-2 Ags on the cell surface in copatching and cocapping experiments with fluorescent Abs (21,22). Also, a 200,000 dalton glycoprotein known as T-200 has been reported to cocap with H-2 on the surface of a thymoma tumor (23). In a different approach, proximity between two different cell surface Ags has been measured by the capacity of Abs to one of the Ags to block access of the second Ag to its own Abs. Using this technique, H-2 molecules were shown to be near neighbors of Thy-1 and Lyt-2 Ags (24,25). In our case, copatching and cocapping of H-2 with gp160 probably is not feasible since the interacting H-2 Ags hardly react with anti-H-2 Abs, and the blocking of H-2 is observed without the need of anti-gp160 Abs.

By adsorption tests with anti-gp160 Abs, obtained in NZB mice immunized with LPC-1 cells, we have so far not been able to detect gp160 on normal spleen, thymus, or liver cells (E. Celis, unpublished results). It is possible, however, that a small proportion of these normal cells could express gp160 on their surface and not be detected by the adsorption assay.

The greater effect of gp160 in blocking access of Abs to the H-2K and H-2D Ags than Abs to some other, non-H-2 Ags, suggests that gp160 and the H-2 Ags interact closely on the cell surface. If gp160 and the H-2 molecules are bound together on the cell membrane, the association is probably due to weak non-covalent bonds, because in NP-40 the molecules are individually precipitated with their respective Abs. Experiments with cross-linking agents are now under way to link covalently any gp160-H-2 complexes that may be present on intact cells.

A cell surface structure that interacts preferentially with the major histocompatibility Ags, blocking access to CTLs, could have survival value for both tumor and normal cells. For instance, the expression of gp160 or similar molecules on the surface of tumor cells could provide a mechanism for tumor cells to escape immune destruction by H-2 restricted CTLs. In addition, during their differentiation some normal cells may express new surface molecules for which the host is not yet tolerant (e.g. idiotypes of surface Ig on developing B cell clones). CTL responses to such self neo-antigens could be avoided if the cell were to mask its major histocompatibility Ags. Regulation of the expression of H-2 Ags, through interaction with gp160, also has the potential advantage that while the antigenic determinants recognized by CTLs (and Abs) are blocked, other parts of the H-2 molecules may still be capable of serving some other function.

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Figure and Table Legends

Figure 1. SDS-PAGE of immunoprecipitated ^{125}I-labeled cell surface proteins from LPC-1 cells. An NP-40 extract of lactoperoxidase-^{125}I-labeled cells was reacted with diverse antisera to non-H-2 Ags. A, DBA/2 anti-LPC-1; B, B10.D2 anti-LPC-1; C, rabbit anti-mouse thymocytes; D, goat anti-Maloney virus. Panel B shows immunoprecipitates obtained with both non-adsorbed serum (○) and serum adsorbed with MOPC-167 cells to remove the anti-PC.1 Abs (●). The position of heavy and light chains from immunoglobulins, indicated by H and L respectively, are molecular weight markers. The LPC-1 cells were harvested 7 days after transplanting $1x10^7$ cells ip.

Figure 2. Comparison of the adsorption of anti-H-2 Abs by early and late LPC-1 cells. Different numbers of early (○) and late (●) LPC-1 cells were tested for their capacity to adsorb C-dependent cytotoxic activity from sera directed against Ags encoded by the $H\text{-}2^d$ complex. A, anti-H-$2K^d$ (H-2.31); B, anti-H-$2D^d$ (H-2.4); C, anti-Ia (A.TH anti-A.TL). The C-dependent cytotoxic activity remaining in the adsorbed serum was measured on ^{51}Cr-labeled MOPC-167 cells. Controls: △, unadsorbed sera; ▲ EL-4 ($H\text{-}2^b$). Broken lines show the area of 50% reduction of cytotoxic titer.

Figure 3 Comparison of the adsorption of different antisera to non-H-2 Ags by early and late LPC-1 cells. Different numbers of early (○) and late (●) LPC-1 cells were tested for their capacity to adsorb C-dependent cytotoxic activity from the following antisera: A,

DBA/2 anti-LPC-1 (anti-PC.1); B, B10.D2 anti-LPC-1 adsorbed with MOPC-167 cells; C, rabbit anti-mouse thymocytes (anti-B220); D, goat anti-Maloney virus (anti gp70, gp100). The C-dependent cytotoxicity remaining in the adsorbed sera was measured on ^{51}Cr-labeled LPC-1 (K) cells (a cultured cell line of LPC-1). Controls; △ unadsorbed sera; ▲ P815; ▩ MOPC-315. Broken line shows the area of 50% reduction of cytotoxic titer.

Figure 4. SDS PAGE of ^{125}I-labeled cell surface proteins from LPC-1, MOPC-315-OPEC, and MOPC-167 cells. Total radiolabel surface protein solubilized in 0.5% NP.40 from LPC-1 (A), MOPC-315-OPEC (B) and MOPC-167 (C) cells. Surface proteins immunoprecipitated with NZB anti-LPC-1 sera (and Staph A) from LPC-1 (D) MOPC-315-OPEC (E) and MOPC-167 (F). The position of heavy (H) and light (L) immunoglobulin chains and gp160 are indicated by the arrows.

Figure 5. Comparison of the adsorption of anti-H-2 and anti-PC.1 Abs by LPC-1, MOPC-315-OPEC, and MOPC-167 cells. Different number of LPC-1 (●), MOPE-315-OPEC (▲) and MOPC-167 (△) cells were tested for their capacity to adsorb cytotoxic activity from anti-H-2^d (B10 anti-B10.D2, A) and anti-PC-1 (DBA/2 anti-LPC-1, B) antisera. The C-dependent cytotoxic activity remaining in the adsorbed sera was measured on ^{51}Cr-labeled MOPC-167 cells. Broken lines show the area of 50% reduction of cytotoxic titer.

TABLE I

Measurement of H-2 Encoded Antigens on the Surface of Myeloma Cells

Cell type[a]	Total cpm[b]	H-2 cpm[c]	H-2/total	Relative H-2[d]
Experiment 1:				
Early LPC-1	1.46×10^7	4105	2.8×10^{-4}	0.40
Late LPC-1	1.38×10^7	3375	2.45×10^{-4}	0.35
MOPC-315-OPEC	1.82×10^7	8379	4.6×10^{-4}	0.65
MOPC-167	3.92×10^6	2843	7.1×10^{-4}	1
Experiment 2:				
Late LPC-1	1.97×10^7	5169	2.6×10^{-4}	0.44
MOPC-315-OPEC	1.36×10^7	6943	5.1×10^{-4}	0.86
MOPC-167	5.98×10^6	3558	5.9×10^{-4}	1

a) Ascites tumor cells were harvested 4 days (early LPC-1) or 12 days (late LPC-1, MOPC-315-OPEC and MOPC-167) after the injection of 1×10^7 cells in per BALB/c recipient.

b) 2×10^7 cells of each tumor were surface-labeled with ^{125}I (and lactoperoxidase) under the same conditions. Cells were solubilized in 0.5% NP-40 and total cpm incorporated into 10% trichloroacetic acid-precipitable material were estimated.

c) The amounts of H-2 Ags were obtained by three sequential immunoprecipitations with anti-$H\text{-}2^d$ sera (B10 anti-B10.D2) and Staph A. The immunoprecipitates were subjected to SDS-PAGE and the number of cpm in the 45,000 dalton region were calculated in each case.

d) This number refers to the amount of surface H-2 Ags relative to MOPC-167 in each experiment.

TABLE II

Stimulation of Secondary Anti-H-2^d CTL by Intact LPC-1 Cells and their Sonicated Membrane Fragments

	Stimulator Cells and Membranes			% Specific ^{51}Cr Release E/T Ratio[c]		
	Source[a]	Protein ($\mu g/10^6$ sonicated cells)	No. of Intact or Sonicated Cells[b] ($\times 10^{-6}$)	100	50	25
EXPT 1	E		0.17	83	86	88
	L		0.17	91	88	87
	EM	9.2	2.5	82	79	77
			5.0	81	75	67
			7.5	80	82	76
	LM	10.1	2.5	18	9	5
			5.0	11	7	2
			7.5	20	11	4
EXPT 2	E		0.17	89	90	91
	L		0.17	86	85	84
	EM	9.5	2.5	58	52	39
			5.0	65	60	46
			7.5	74	72	49
	LM	12.5	2.5	21	15	10
			5.0	30	25	15
			7.5	39	30	20
	S-EM	7.5	2.5	55	46	29
			5.0	50	42	30
			7.5	55	46	33

		2.5	21	15	10
LM	12.5	5.0	30	25	15
		7.5	39	30	20
		2.5	55	46	29
S-EM	7.5	5.0	50	42	30
		7.5	55	56	37
		2.5	43	30	21
S-LM	8	5.0	61	51	37
		7.5	72	69	57
——		0	7	5	4

[a]Spleen cells from primed BALB.K mice (one injection of $1x10^7$ BALB/c spleen cells, 30 days earlier) were stimulated in a 5-day in vitro culture with irradiated (10,000 RADS) intact early (E) or late (L) LPC-1 cells. Sonicated membranes from early and late cells (EM and LM, respectively) were also used. In some cases, the early and late LPC-1 cells were treated with subtilisin (0.2 mg/ml, 30 min, room temperature) before sonication and partial purification of the membrane fragments (S-EM, S-LM).

[b]Number of tumor cells used as intact cells, or for sonication to obtain membrane fragments for use as stimulators per $7x10^6$ responder (BALB.K) cells.

[c]The activity of the responder cell population was measured for its capacity to lyse ^{51}Cr-labeled P815 ($H\text{-}2^d$) cells in a 4 hr assay, at 3 different effector to target ratios (E/T).

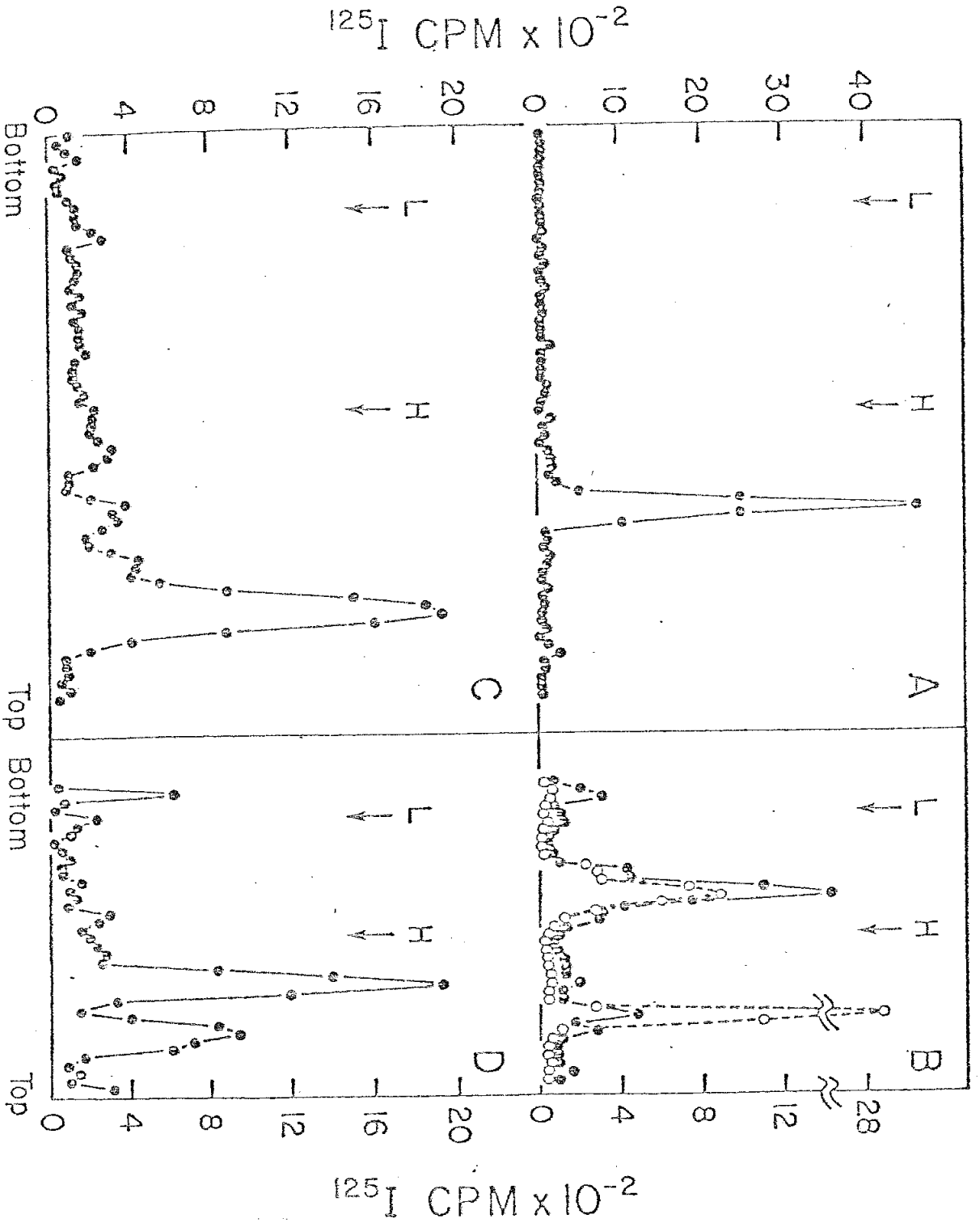
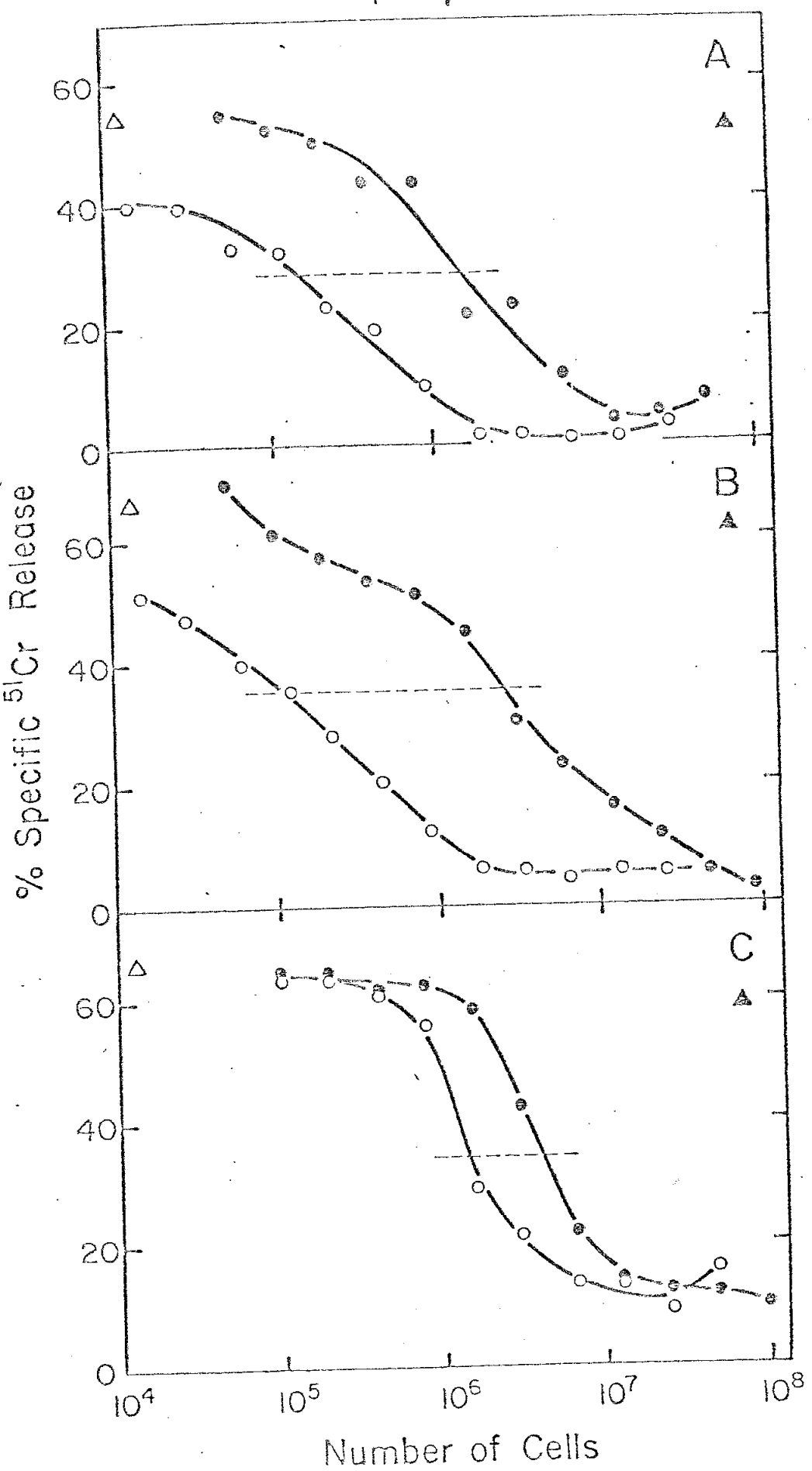
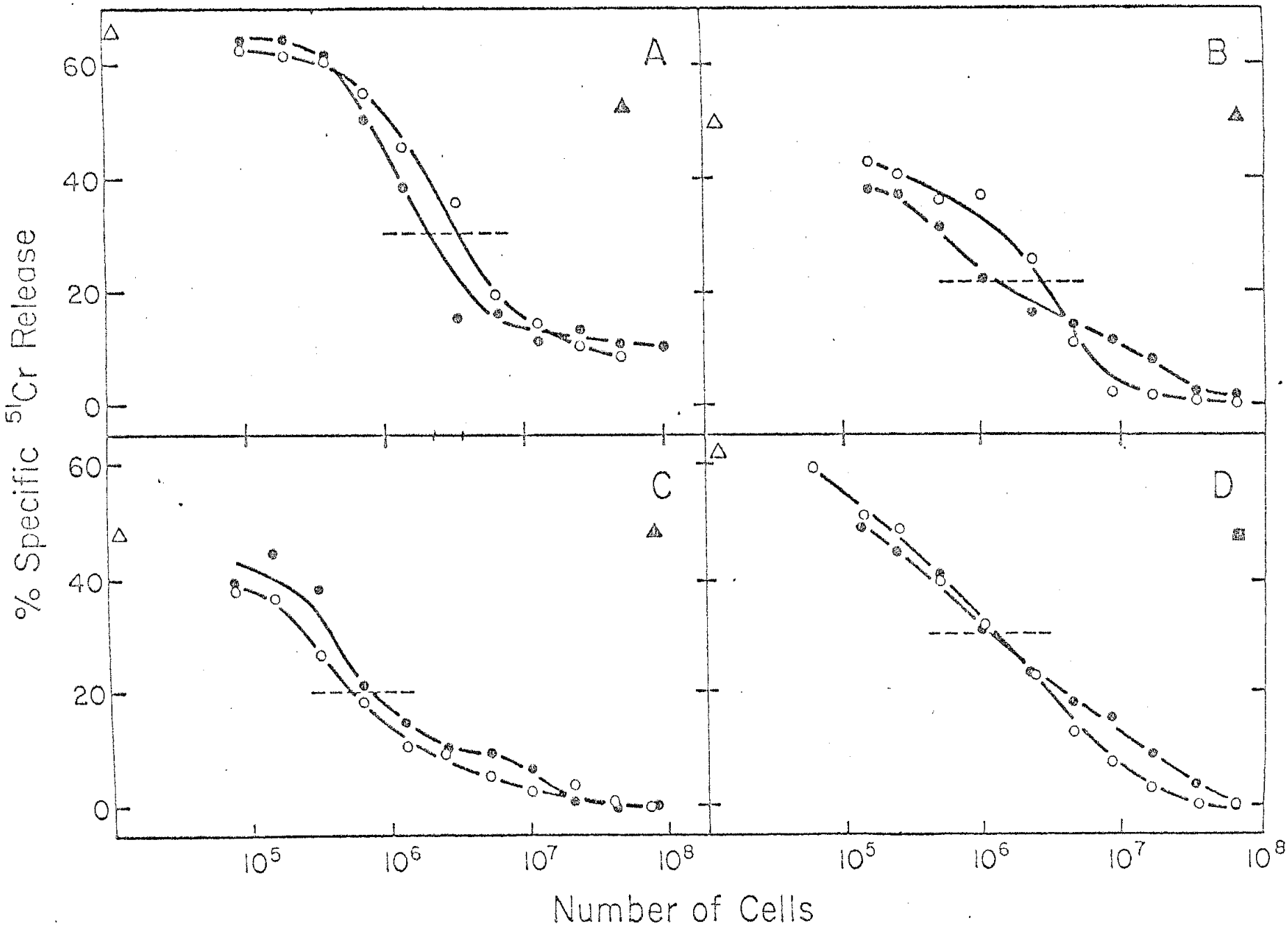

^{125}I CPM x 10^{-2}
0
4
8
12
16
20
Bottom
Top
C
D
0
10
20
30
40
A
B
L
H
0
4
8
12
28
^{125}I CPM x 10^{-2}

FIG. 1

FIG 2


A
B
C
60
40
20
0
% Specific ^{51}Cr Release
10^4
10^5
10^6
10^7
10^8
Number of Cells


A
B
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60
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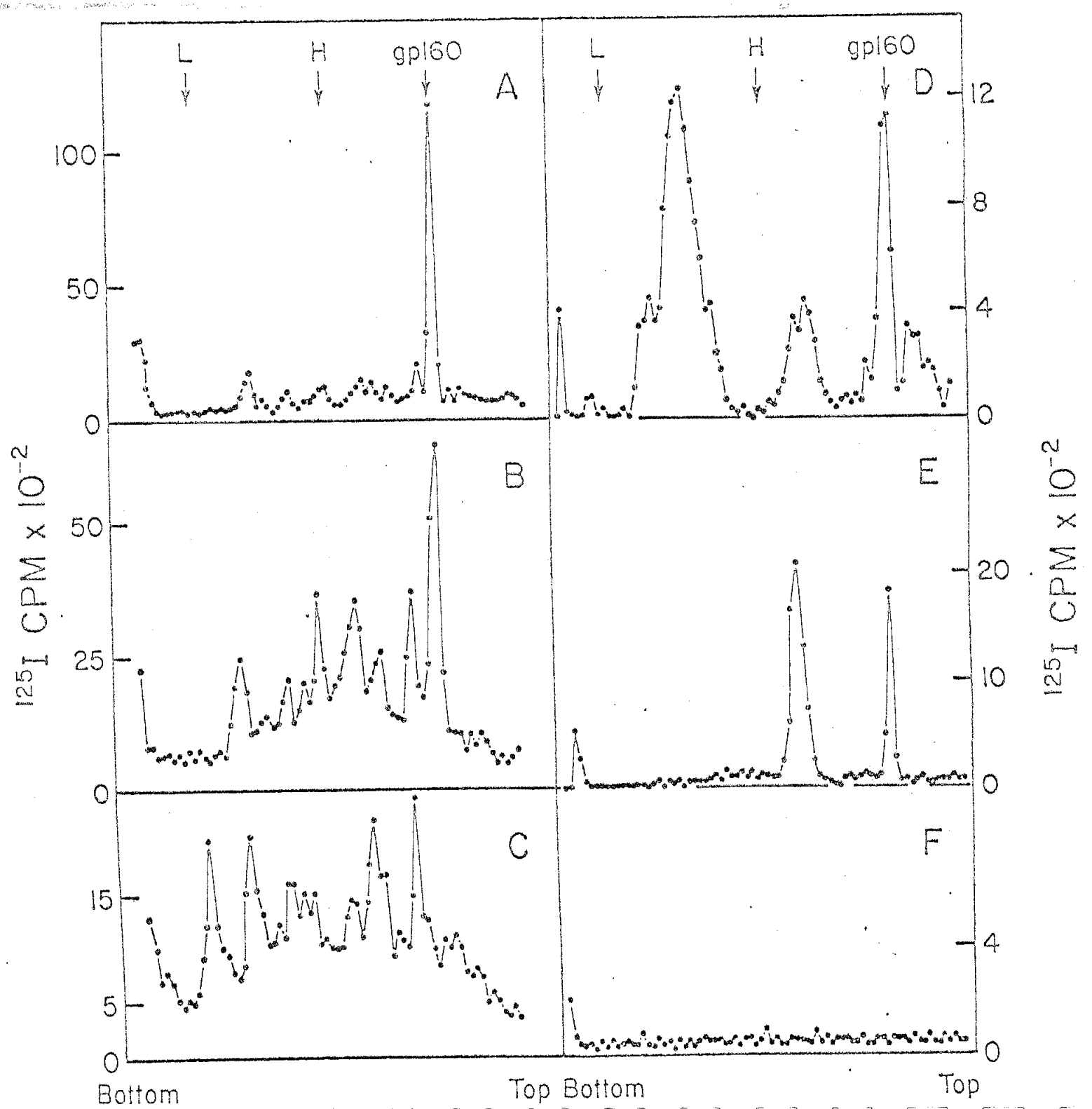
20
0
% Specific ^{51}Cr Release
10^5
10^6
10^7
10^8
Number of Cells


L
H
gp160
A
B
C
D
E
F
100
50
0
50
25
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15
5
0
12
8
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^{125}I CPM x 10^{-2}
^{125}I CPM x 10^{-2}
Bottom
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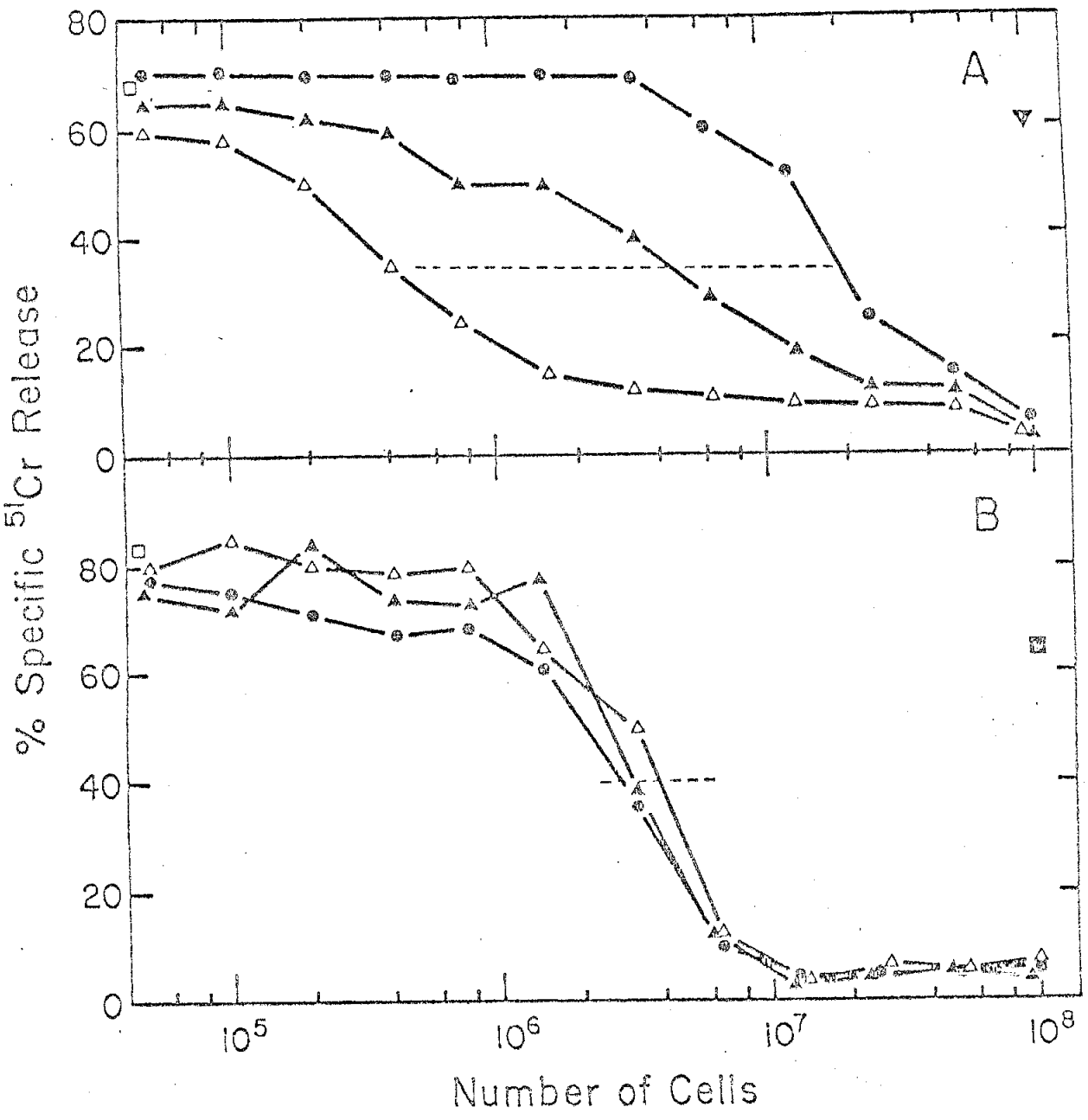
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B
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60
40
20
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% Specific ^{51}Cr Release
10^5
10^6
10^7
10^8
Number of Cells

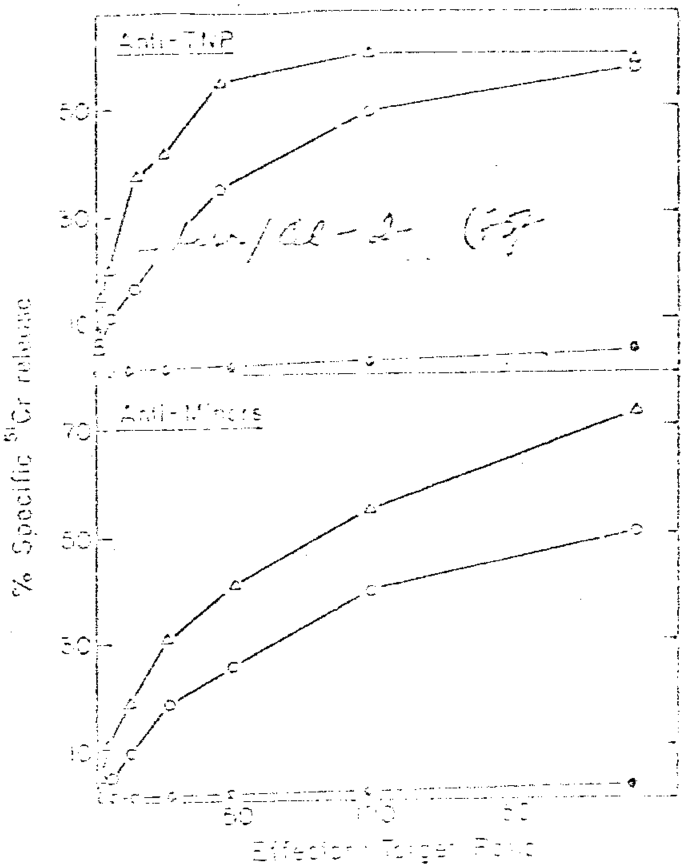

FIG. 2. Susceptibility of early and late LPC-1 cells to lysis by CTLs directed against non-H-2 cell surface antigens. The ^{51}Cr-labeled target cells were early ([illegible]) and late ([illegible]) LPC-1 cells and BALB/c Con A blasts ([illegible]). In the upper panel, the effector cells were BALB/c spleen cells stimulated with Tnp-BALB/c spleen cells (anti-Tnp CTLs), and the targets were all Tnp-modified. In the lower panel, the effector cells were B10.D2 CTLs elicited with BALB/c spleen cells (anti-minor histocompatibility Ags), and the targets were not Tnp-modified. The symbols for the targets are the same in the upper and lower panels [36].

and that other proteases with more restricted specificities, such as thrombin and Staphylococcus [illegible] protease, failed to produce this effect [38]. Trypsin treatment of the late LPC-1 cells also increased their capacity to absorb anti-H-2[d] Abs to an extent equal to that of the early cells (Fig. 3).

In order to test if a trypsin-sensitive protein might have been blocking the accessibility of the H-2 molecules to both CTLs and Abs, an analysis of the cell surface proteins of the early, late, and trypsin-treated late LPC-1 cells was performed [38]. The surface proteins of the early and trypsin-treated late LPC-1 cells

DISCUSION GENERAL

Nuestro interés en el estudio de la reacción de los receptores de las células inmunes con sus ligandos radica en que la función efectora de estas células -ya sea la producción de Acs por los linfocitos B o la función ayudadora, supresora o citolítica de los linfocitos T-, es disparada por dicha reacción. Por lo tanto, durante un reto antígenico -como durante el surgimiento de una infección o de un tumor-, el desarrollo de una respuesta inmune eficaz que otorgue resistencia al huésped dependerá de que el Ag logre alcanzar y fijarse a los receptores de las células inmunes. De este hecho se deriva la importancia del estudio de los factores que pueden de algún modo modificar y regular la reacción ligando-receptor. Algunos de estos factores han sido estudiados y discutidos por separado en los artículos aquí presentados. A continuación se intentará llevar a cabo una discusión general tomando en cuenta lo que a nuestra manera de pensar nos parece de mayor relevancia de cada uno de los siete artículos y tratando de integrar toda esta información en un mismo cuadro teórico que de ninguna manera se acerca o intenta ser una descripción detallada de la reacción ligando-receptor.

En primer lugar, en un modelo bioquímico consistente en Acs purificados acoplados a Sefarosa capaces de reaccionar con Ags solubles radioactivos, que simula la reacción del Ag con los receptores de los linfocitos B se encontró que la valencia del Ag juega un papel principal principal en la reac-

-ción determinando su constante de afinidad aparente (Ka). Es decir, que a mayor valencia antigénica, mayor Ka de la reacción ligando-receptor (10). Estos resultados están de acuerdo con algunas observaciones realizadas in vivo sobre la respuesta inmune donde se reporta que Ags de alta valencia son mayormente inmunogénicos, toleragénicos e inductores de memoria inmunológica que Ags con baja valencia (19, 20). Estos resultados fueron explicados por medio de la relación directa que existe entre la valencia del ligando y la Ka propuesta por Klotz (21) que supuestamente se debería aplicar a la reacción de los Ags con los receptores celulares. Los resultados obtenidos con nuestro modelo bioquímico sirven para confirmar dicha suposición.

Inesperadamente se observaron en las gráficas de Scatchard (11) trazos ascendentes en la porción de bajas concentraciones de ligando que también parecen encontrarse relacionados en su magnitud con la valencia del Ag (10). Estas anomalías en las gráficas de Scatchard solo pudieron ser explicadas por medio de interacciones positivas (o cooperatividad) entre los sitios activos de los receptores. La simulación de la reacción de fijación ligando-receptor por medio de un modelo matemático computacional donde se estudiaron parámetros tales como la heterogeneidad de los sitios activos, fijación inespecífica del ligando e interacciones tanto positivas como negativas entre los sitios activos, reveló que únicamente cuando hay cooperatividad se producen estos trazos ascendentes en las gráficas de Scatchard (10).

Para estudiar el efecto que puede tener la presencia de Acs (solubles) en la fijación de ligando a los receptores celulares se utilizó el mismo modelo bioquímico. Este caso particular trataría de simular una respuesta inmune secundaria, donde el individuo esté en contacto por segunda vez con el mismo Ag y se encuentran presentes en el sistema Acs que fueron elaborados durante el primer reto antigénico. Como se esperaba, cantidades altas de Acs compiten con los receptores por el ligando, ocupando determinantes antigénicos, disminuyendo la valencia real del Ag y tal vez también creando impedimentos estéricos en la reacción (12). Sin embargo, se observó que cantidades bajas de Acs incrementaron la fijación de ligandos multivalentes a los receptores celulares. Este sinergismo se presentó también aún cuando la especificidad de los Acs y la de los receptores era distinta, pero necesariamente ambas especificidades debían estar presentes en la misma molécula de ligando (12). Por medio de un experimento de doble marca, utilizando Acs radioiodinados se demostró que el sinergismo observado probablemente se debe a la creación de polímeros formados por Ags y Acs, los que además de elevar la valencia real del ligando, hacen posible que un receptor celular pueda ser ocupado por mas de una molécula de Ag (12). El papel regulatorio de los Acs en la respuesta inmune -a travez de sinergismo o competencia de la reacción ligando-receptor- se propone en este trabajo (12) simulando el transcurso de un reto antigénico en un modelo computacional.

En esta simulación se observó que en los inicios de la respuesta inmune los primeros Acs actuan sinérgicamente y mas adelante, al aumentar la cantidad de éstos, lo hacen competitivamente. Algunos fenómenos descritos en la literatura como el llamado "efecto del acarreador", donde se observa que una respuesta de Acs a un hapteno puede ser significativamente mayor si se inmuniza previamente con la proteína acarreadora (22, 23), pueden ser explicados en parte por la presencia de Acs anti-acarreador, que actuen como en nuestro modelo, sinérgicamente. La aparente ventaja que presentan los receptores sobre los Acs en la fijación del ligando, probablemente se deba al microambiente que otorga la fase sólida a esta reacción y explica como las moléculas que son antigénicas llegan a alcanzar a los receptores de las células inmunes aún existiendo sistemas competidores solubles (12). Es importante señalar además que según nuestro modelo bioquímico, las clonas inmunológicas no son del todo independientes unas de otras sino que ellas mismas o sus productos pueden regularse entre sí cuando los Ags poseen mas de un tipo de determinante antigénico, lo que generalmente ocurre.

Con el objeto de estudiar la reacción de los receptores de los linfocitos T con sus ligandos, se escogieron a los linfocitos T citotóxicos (LTCs) ya que son relativamente fáciles de obtener y su función efectora se mide directamente sobre sus células blanco. La interacción de estas células líticas con células tumorales (como células blanco) tiene

además el atractivo de que estudia el fenómeno inmune de las enfermedades neoplásicas.

Las observaciones realizadas con microscopía indican que las interacciones de los LTCs con las células tumorales son muy íntimas, dinámicas y poco específicas. El contacto íntimo entre los LTCs y sus células blanco se observó claramente al microscopio de barrido de electrones, donde en imágenes tridimensionales las células efectoras parecen envolver los procesos citoplásmicos de las células blanco y también tienden a insertarse los LTCs entre las células blanco y el sustrato sólido donde crecen y se encuentran adheridas (13). El gran dinamismo de las células T es aparente al observar cómo estas células recorren toda la superficie de las células blanco, utilizando movimientos amiboides y con velocidades a veces hasta de 20 um/min. Por último, la falta de especificidad de estas interacciones parece indicar que este proceso precede a la reacción receptor-ligando y probablemente se trate de un mecanismo de exploración de la estructura antigénica de la superficie de las células blanco. Por otro lado, en los casos donde las células blanco sí poseían los Ags a los que los LTCs se encontraban dirijidos, se observaron en varias ocasiones ejemplos de citólisis (13).

El papel esencial de los productos del CMH, H-2K y H-2D en la interacción de los LTCs con células tumorales es evidente por los resultados obtenidos con algunos mielomas de

la cepa BALB/c de ratón (14-17). Los mielomas LPC-1 y MOPC-315-OPEC se vuelven resistentes a la lisis por LTCs conforme crecen en la cavidad peritoneal del ratón, enmascarando sus moléculas H-2K y H-2D por medio de una glicoproteína de 160,000 daltones (gp160) que los tumores mismos sintetizan (14-16). Esta resistencia a la lisis por LTCs desapareció cuando se trataron a las células mielomatosas con algunas proteasas que remueven a gp160 de su superficie (16). La presencia de gp160 en la superficie de algunas células tumorales de ratón con su efecto claramente protector a la destrucción inmune, podría tratarse de uno de los mecanismos de evasión inmunológica de las enfermedades neoplásicas, y siendo los procesos tumorales uno de los problemas mundialmente mas serios de la salud, nos parece de mérito suficiente seguir con estos estudios y analizar mas detalladamente la frecuencia de gp160 en tumores murinos, buscar su presencia o la de una glicoproteína similar en tumores humanos y estudiar el mecanismo de interacción de gp160 con los productos del CMH. Algunos estudios preliminares señalan que gp160 no es una glicoproteína común en otros tumores o en células normales del ratón. Sin embargo, ha sido posible en dos diferentes ocasiones inducir la expresión de gp160 en células de mieloma de BALB/c, seleccionando sublineas de crecimiento acelerado y no productoras de inmunoglobulinas. Nos interesa determinar además, si gp160 se encuentra codificada por el genoma murino o si es un producto de algún virus no lítico que haya infectado a las células de mieloma.

El mecanismo molecular de interacción entre gp160 con H-2K y H-2D será explorado utilizando vesículas sintéticas de membranas o liposomas, con preparaciones puras o semipuras de proteínas de superficie celular y estudiando la capacidad de éstas de inducir una respuesta a LTCs cuando se encuentra presente o no gp160. Además nos gustaría determinar si la porción polipeptídica o de carbohidratos de esta glicoproteína es la responsable del bloqueo de las moléculas H-2 a los linfocitos T. La regulación de la síntesis de gp160 en estas células tumorales será estudiada por medio de un sistema de traducción in vitro de RNAm total, determinando así la cantidad y velocidad de síntesis de gp160 y comparándola con otros Ags de superficie a distintas etapas de crecimiento de estos tumores en el ratón (17,18).

Por último, nos proponemos estudiar varios tumores de origen humano, particularmente mielomas para determinar si alguno posee una proteína similar o equivalente a gp160 que interaccione con los productos del complejo HLA, produciéndose resistencia a la lisis por LTCs. La presencia de alguna glicoproteína de este tipo en tumores humanos sería altamente significativa ya que, como se mencionó anteriormente, podría ser éste un ejemplo de como algunos tumores logran evadir a la respuesta inmune en el hombre (24).

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