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HUMAN T AND B LYMPHOCYTE MIGRATION UNDER AGAROSE: DIFFERENCES IN THE CHARACTERISTICS OF MIGRATING CELLS AND MIGRATION PATTERNS

ヒトT，Bリンパ球のアガロース下遊走，
遊走細胞及び遊走像の特性における差異

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SUMMARY

The morphologic and migration characteristics of separated human peripheral blood T and B lymphocytes were evaluated by means of an agarose plate technique. The motile T cells migrated in a tightly packed and regularly arranged monolayer, while the B cell monolayer appeared much more disorganized with the cells forming a loose spongelike network. Fixed and stained preparations following 2-3 days of incubation showed that the T cells maintained mature lymphocyte features throughout the period of migration. Although many of the B cells were indistinguishable from the T cells, many were irregular in size and shape with monocytic characteristics. It is suggested that the differences in T and B cell in vitro migration patterns may reflect fundamental differences in the mechanisms of locomotion for these cells.

INTRODUCTION

A modification of the agarose plate technique, used for the study of granulocyte and monocyte migration,[1,2] recently has been applied to the study of lymphocyte motility.[3] In this report attention is focused on the random migration patterns and morphologic characteristics of migrating subpopulations of human lymphocytes. In contrast to previous studies where observations were made for periods of up to several hours,[4-12] each of our studies extended over a period of several days. Emphasis in this report is placed on the morphologic characteristics of migrating T and B lymphocytes in the absence of any known mitogenic substances.

要 約

アガロース平板法を使用して，ヒトの末梢血液から分離したT，Bリンパ球の形態及び遊走上の特性を評価した．遊走T細胞は互いに密集し，規則的に配列する単一層を作って遊走するが，一方，B細胞の単一層は海綿様の粗い網のようにはるかに不規則な様相を呈していた．2－3日間培養後，固定し染色した標本では，T細胞は全遊走期間中成熟リンパ球の特性を持続することが判明した．T細胞と区別し得ないB細胞も多数あるが，その多くは単球の特性を持ち，形と大きさが不整であった．T，B細胞の試験管内遊走像の違いは，両細胞の遊走機序における根本的な差異を反映するものと示唆される．

緒 言

顆粒球及び単球遊走検査に使用されたアガロース平板法[1,2]の変法を，最近，リンパ球遊走検査に応用した．[3] 本報では，ヒト遊走リンパ球subpopulationの不規則遊走像並びに形態的特性に注目した．従来の研究では観察期間が数時間であったのに対し，[4-12] 我々はそれを数日間に延長した．本報では，既知の細胞分裂刺激剤を用いずに遊走させたT，Bリンパ球の形態的特徴に重点を置いた．

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MATERIALS AND METHODS

Agarose A-45 (agarose) was purchased from Nakarai Chemicals, Ltd., Kyoto, Japan; Medium RPMI-1640 (RPMI) from Nissui Seiyaku Co. Ltd., Tokyo, Japan; pooled human serum as Pentex (PHS) from Miles Laboratories, Inc., Elkhart, Indiana; and fetal calf serum (FCS) from Grand Island Biological Co., Grand Island, New York. Sheep red blood cells (SRBC) were purchased from the Japanese Biological Materials Center, Tokyo, Japan; and Amphotericin B as Fungizone from E. R. Squibb and Sons, Inc., Princeton, New Jersey. Plastic petri dishes, 60×15 mm, were obtained from Falcon, Oxnard, California; Ficoll-400 (Ficoll) from Pharmacia, Uppsala, Sweden; and Conray 400 (Conray) from Daiichi Seiyaku Co., Tokyo, Japan.

The agarose technique for the study of lymphocyte migration has been reported previously in some detail.[3] Human granulocytes and lymphocytes in heparinized venous blood were separated according to the Boyum[13] method using sterile technique. T and B cell separation by SRBC rosetting of T cells and density centrifugation was accomplished by a modification of the method of Greaves and Brown.[14] In order to reduce monocyte contamination in some B cell preparations glass adsorption was used. The B cells were suspended in 3.0 ml of RPMI in a glass culture flask and were incubated for 30 minutes in a humidified 5% CO_2 incubator at 37C. The nonadherent cells were drawn off by pipette and washed twice in balanced salt solution. All cell preparations were finally suspended in RPMI to a concentration of 1×10^6 cells per 10 μl. Viability of the cells following washing was more than 97% by trypan blue exclusion.

Monocyte contamination was evaluated by nonspecific esterase staining.[15] Approximately 15%-20% of cells in the unadsorbed B cell preparations were estimated to be monocytes; glass adsorption reduced this figure to less than 5%. Fewer than 1% of cells in the T cell preparations were identified as monocytes by means of the nonspecific esterase stain studies.

An agarose mixture containing antibiotics and other nutrients was prepared according to the method previously described.[3] Five ml of the agarose-nutrient solution mixture was poured into each petri dish. They were hardened by refrigeration at 4C. Just prior to use, three

材料及び方法

使用材料及びその購入先は下記のとおりである：アガロースA-45（アガロース）－半井化学薬品，京都；RPMI-1640培地（RPMI）－日水製薬，東京；Pentexとしてプールしたヒト血清（PHS）－Miles Laboratories, Inc., Elkhart, Indiana；ウシ胎児血清（FCS）－Grand Island Biological Co., Grand Island, New York；ヒツジ赤血球（SRBC）－日本生物材料センター，東京；Fungizoneとして Amphotericin B－E. R. Squibb and Sons, Inc., Princeton, New Jersey；プラスチック・ペトリ皿 60×15mm －Falcon, Oxnard, California; Ficoll-400（Ficoll）－Pharmacia, Uppsala, Sweden；及びConray 400（Conray）－第一製薬，東京．

リンパ球遊走検査用のアガロース技法については，前報で詳述した．[3] 滅菌技法使用の Boyum 法[13] に従って，ヒトのヘパリン添加静脈血から顆粒球及びリンパ球を分離した．Greaves 及び Brown の変法[14] に従い，T細胞と SRBC のロゼット形成及び密度遠心沈殿によりT，B細胞の分離を行った．B細胞標本に生じる単球の混入を減少させるため，ガラス吸着を使用した．ガラス培養フラスコ中の3.0ml の RPMI にB細胞を懸濁し，37℃の高湿 5 %CO_2 恒温器で30分間培養した．付着しなかった細胞をピペットで取り出し，平衡化食塩水で２回洗浄した．最後に，全細胞標本を RPMI に懸濁し，10μl 当たり細胞数 1×10^6 の濃度にした．洗浄後の細胞の生存力をトリパン・ブルー排除検査で測定した結果は97%以上であった．

非特異性エステラーゼ染色によって，[15] 単球混入を測定した．非吸着B細胞標本では細胞の約15%－20%が単球と推定された．ガラス吸着でこの率は５%未満に減少した．非特異性エステラーゼ染色により，T細胞標本で細胞の１%未満が単球であると確認された．

前報で述べた方法[3] に従って，抗生物質及びその他の養液を含むアガロース混合液を用意した．アガロース－養液混合液を各ペトリ皿に注ぎ，４℃で冷蔵して固めた．使用直前に，ステンレス・スチールの型で

circular wells, each 3.0mm in diameter and 4.0mm apart in a straight line were cut with a stainless steel template. The core of agarose in the center of each well was carefully removed by gentle suction through a Pasteur pipette, care being taken not to disturb the adherence of the adjacent agarose-dish interface.

A 10μl aliquot (1×10^6 cells) of T, B, or monocyte adsorbed B cells suspended in RPMI was carefully placed in each of the lateral wells by means of a capillary pipette. The middle well, reserved for the introduction of substances which may influence migration, was not used for these experiments. Sufficient numbers of cells were obtained from each donor so that 5-7 different plates containing either T, B, or monocyte adsorbed B cells could be prepared. The plates were incubated at 37C in a humidified CO_2 incubator for periods up to 7 days. Plates were removed at 1-2 day intervals and fixed by covering the agarose with Carnoy's solution following which the agarose was removed and the cells stained with either Giemsa or Wright-Giemsa. This procedure was followed in order to make histological comparisons among different individuals, as well as in the same individual over time. In addition, plates were periodically examined during incubation to observe the morphologic features of actively motile cells. Migration distances in fixed preparations were measured by means of a table top 35mm slide projector with a ground glass screen which provided 20-fold magnification. The distance between the edge of the agarose well and the leading edge of the migrating cells was measured in four directions for each of the two wells on a plate. The final migration distance which was recorded for each study consisted of the average of these values.

In order to differentiate B lymphocytes from monocytes, even in monocyte adsorbed preparations, the cells of some of the preparations were stained for nonspecific esterase following 3 days of migration under agarose. The modified method used for nonspecific esterase staining of the migration preparations has previously been described.[16]

RESULTS

The lymphocytes in both the T and B cell preparations migrated outwardly from the well

アガロース上に一直線に4.0mm 間隔で直径3.0mm の円形試料孔を3個くりぬいた．周囲のアガロースとペトリ皿の付着面を壊さないように注意しながら，パスツール・ピペットで各試料孔中心部のアガロース柱状塊を静かに吸引して除去した．

RPMI に懸濁した10μl （1×10^6 細胞）のT，Bないしは単球吸着B細胞を毛細管ピペットで外側の各試料孔に注意深く注入した．中央の試料孔は，遊走に影響を及ぼす可能性のある物質を注入するために残したが，今回の検査では使用しなかった．各供血者から十分な数の細胞を入手したので，T，Bないしは単球吸着B細胞を含む平板を5－7枚作成することができた．これらの平板を高湿 CO_2 恒温器に入れ，37℃で最高7日間まで培養した．1－2日間隔で平板を取り出し，アガロースにCarnoy 液を注ぎ固定した後，アガロースを除去し，細胞を Giemsa 又は Wright-Giemsa で染色した．この操作で個人間の，及び，同一個人の経時的な組織学的比較を行った．更に，培養中定期的に平板を検査し，活発に遊走する細胞の形態的特徴を観察した．すりガラス製スクリーン付の卓上型35mm スライド・プロジェクターを用い20倍の倍率で，固定標本の遊走距離を測定した．1枚の平板上の2個のアガロース試料孔それぞれについて4方向で試料孔の端から遊走細胞の先端までの距離を測定した．最終的な遊走距離はこれらの値の平均から算出し，各検査ごとに記録した．

Bリンパ球と単球を区別するために，単球吸着の場合でも，若干の標本ではアガロース下で3日間遊走後の細胞に非特異性エステラーゼ染色を行った．遊走標本に用いた非特異性エステラーゼ染色の変法は前報で説明した．[16]

結　果

T，B両細胞標本中のリンパ球は，各平板の底面の

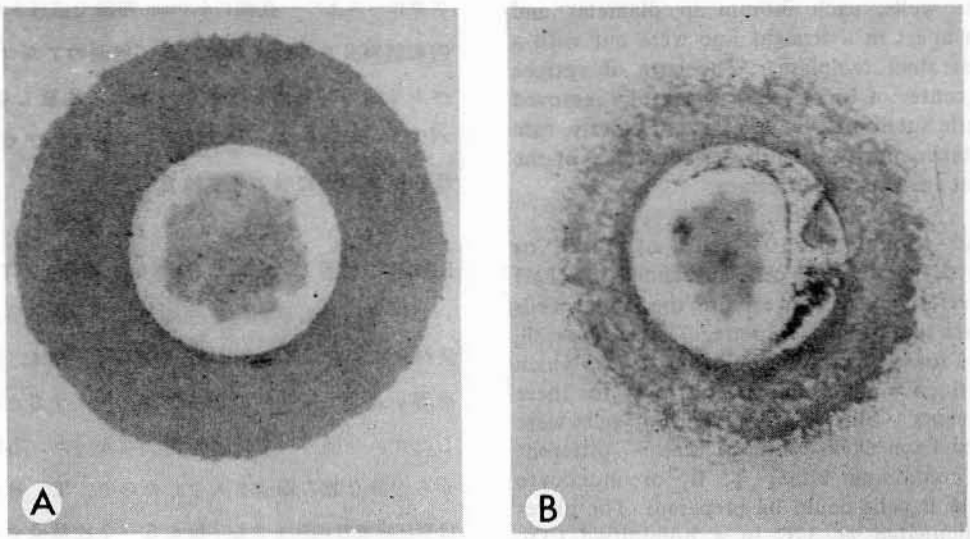

Figure 1 Stained lymphocyte migration preparations following 3 days of incubation. The center circles represent the wells from which the cells have migrated radially. (A) T lymphocyte preparation (B) B lymphocyte preparation (×10).

図1　3日培養後のリンパ球遊走染色標本．中央の円は，細胞の放射状遊走の起点となる試料孔を示す．（A）Tリンパ球標本（B）Bリンパ球標本（10倍）．

as a monolayer on the bottom of each plate at the agarose-plastic surface interface. Detectable outward migration occurred only after several hours and then proceeded quite uniformly during the next few days producing visible opaque rings of cells around the wells in a fashion similar to that observed with granulocytes after a few hours (Figure 1).[1,2] The characteristics of the migration patterns of the T lymphocytes, as observed in the stained preparations, varied little from one individual to another although there were modest variations in migration distances. T lymphocytes appeared as a monolayer of contiguous cells in close apposition to one another. The leading edge of the out-migrating cells usually was sharply demarcated, smooth and regular, but some preparations presented scalloped or pedunculated borders. In contrast, the B cell monolayers, both monocyte adsorbed and nonadsorbed, were much more loosely arranged with the leading edges being fragmented, frayed, and irregular in appearance. Moderate numbers of cells invariably were located out beyond the leading edges of the B cell preparations in contrast to the T cell preparations where such occurrences were rare (Figure 2). The precise measurement of the B cell migration distance sometimes was difficult

アガロースとプラスチック表面との界面を単一層として試料孔から外方に遊走した．検知可能な外方遊走が始まったのは数時間経ってからであった．その後，2，3日間は全く一様な遊走が続き，2，3時間遊走後の顆粒球で観察されるのと同様に，試料孔の周囲に不透明な細胞環が認められた（図1）．[1,2] 染色標本で見られるように，Tリンパ球遊走像の特性には個人差はほとんどないが，遊走距離に僅かの差があった．Tリンパ球は，互いにぴったりとくっついて並んだ細胞の単一層として遊走した．外方に遊走する細胞の先端は通常，鮮明な境界を有し，滑らかで規則的であったが，中には，波状ないしは茎状の境界を示す標本もあった．これと対照的に，B細胞の単一層は単球吸着，非吸着いずれの標本の場合にも，T細胞よりずっと疎に配列し，その先端はばらばらにほつれ，不規則な様相を呈していた．B細胞標本の場合，相当数の細胞が常に先端外にあったが，T細胞標本ではこのような現象はまれであった（図2）．B細胞標本の場合，外縁が不鮮明なため，遊走距離の正確

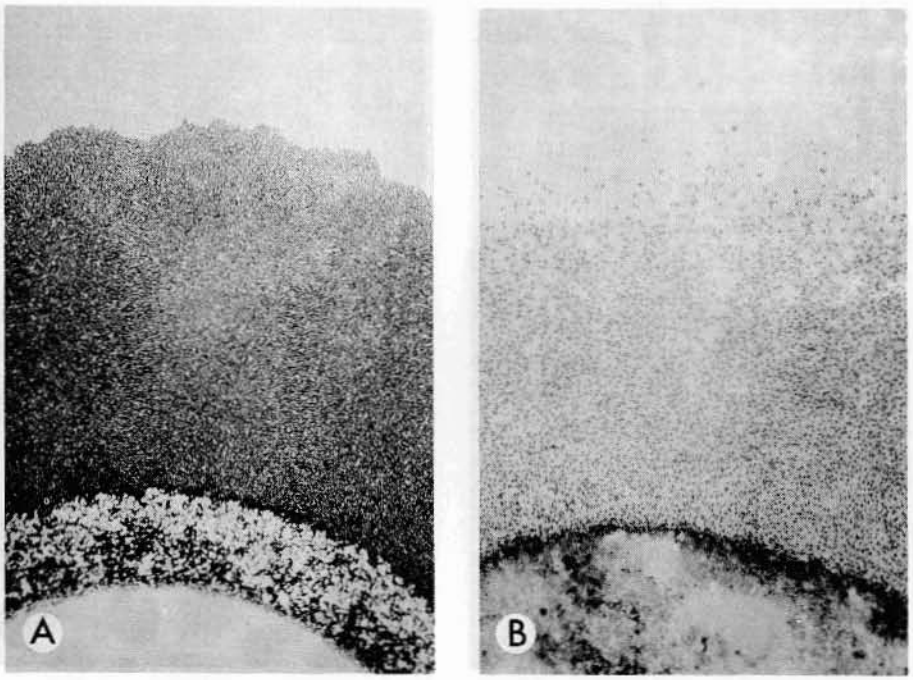

Figure 2 Portion of stained lymphocyte migration preparations following 3 days of incubation. Note the intact, even advancing edge of the T cell preparation (A) in contrast to the characteristic frayed and indistinct leading edge of the B cells (monocytes adsorbed) (B) (×35).

図2　3日培養後のリンパ球遊走染色標本の一部．（A）T細胞標本の先端部が損われず平担であるのに対し（B）B細胞（単球吸着）の特徴的なほつれた形の不明瞭な先端部に注目（35倍）．

to obtain due to the absence of a sharp outer margin.

The migration distances of the T and B cells were quite similar for the first 2-3 days. Usually they were in the range of 1-2mm. During the next several days there was little evidence of continued outward migration of the T cells. Further outward migration of the B cells during the next few days was erratic and inconsistent from one individual to another. There was progressively greater dispersion of marginal cells. The unadsorbed B cell preparations were even more spongy and frayed in appearance. Many individual or clustered monocytoid cells extended well beyond the advancing edge.

The morphologic appearance of Wright stained cells in the T cell preparations was remarkably uniform during the first 2-3 days of observation (Figure 3). Well over 90% of the cells had the characteristics of small to medium sized lymphocytes. About half had elongated cytoplasmic

な測定が困難なこともあった．

T，B細胞の遊走距離は，最初2，3日間はかなり類似していた．通常，その距離は1－2mmの範囲であった．T細胞の場合，その後数日間外方遊走が継続する所見はほとんど認められなかった．B細胞では，その後2，3日間の外方遊走は無秩序で，個人差があった．外縁の細胞の分散状態は漸増していった．非吸着B細胞標本は一層海綿状となり，ほつれた様相を呈し，多数の単球様細胞が単独であるいは密集して前進縁を越え遊走した．

T細胞標本中のWright染色細胞の形態は，観察最初の2，3日間は極めて一様であった（図3）．90%をはるかに超える細胞が小型ないし中型リンパ球の特性をそなえていた．約半分が，無顆粒状で均質な，

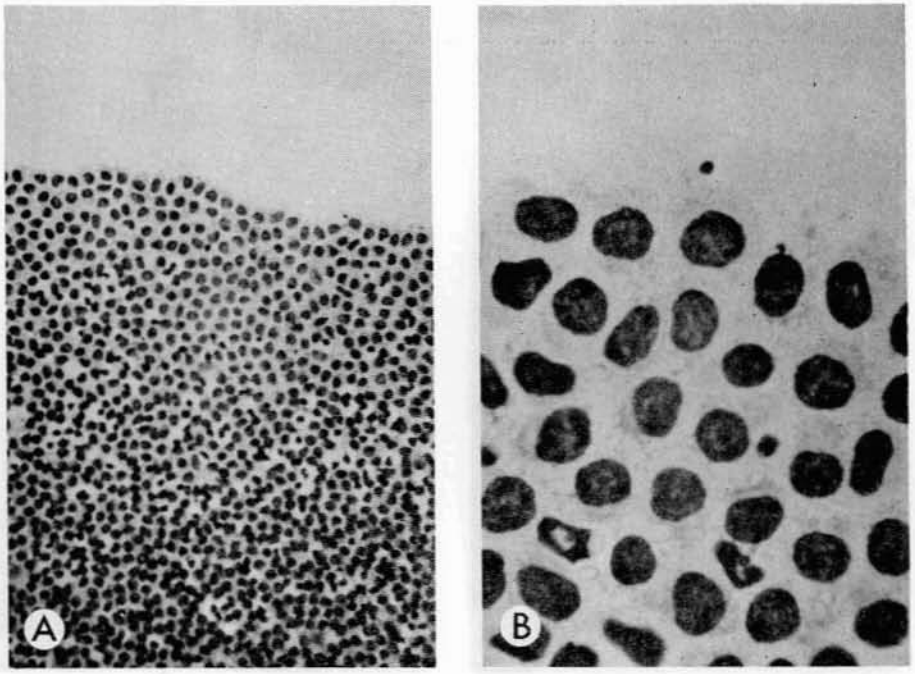

Figure 3 Stained T cell preparation following 3 days of incubation. Note the typical appearance of small and medium sized mature lymphocytes. A portion of the advancing edge is shown (A×100, B×400).

図3　3日培養後のT細胞染色標本．小型及び中型の成熟リンパ球の典型的な外観に注意．先端の一部を示す（A 100倍，B 400倍）．

tails with agranular, homogeneous light blue staining cytoplasm and oval or round nuclei. The tails of the cells pointed in various directions with no more than 30% pointing towards the point of origin at any one time. Moderate cytoplasmic vacuolization was observed. Most of the remaining lymphocytes were round or oval in shape without a tailed configuration. The cytoplasmic borders of most cells were quite sharply demarcated, especially in the outer several layers of cells. The nuclear chromatin of the lymphocytes had a uniform moderately dark blue appearance. No nucleoli were observed and there was little evidence of nuclear chromatin clumping. No mitoses were observed. The cells along the outer edge tended to be more clearly defined, slightly larger, and more irregular in shape than the other lymphocytes. Lymphocyte size for all lymphocytes on these preparations was estimated to range from 5-10μ. Occasional monocytoid cells and polymorphonuclear leukocytes were observed. From 1%-3% of the cells in the preparations were condensed, pyknotic or karyorrhectic in appearance.

淡青染細胞質及び楕円形又は円形の核を持つ長い細胞質尾部を有していた．細胞尾部は様々の方向に向いており，遊走原点を指向するものは常時30%に過ぎなかった．中等度の空胞形成が観察された．残余のリンパ球はほとんど円形あるいは楕円形で，尾部がなかった．大部分の細胞の細胞質境界は特に細胞の外側の数層で全く鮮明であった．リンパ球の核染色質は一様に，紺色で核には認められず，核染色質凝集の所見もほとんどなかった．有糸分裂は観察されなかった．外縁沿いの細胞は他のリンパ球よりも境界鮮明で，少し大きく，形が不規則であった．これら標本中のリンパ球の大きさは5μ-10μであると推定された．単球様細胞及び多形核白血球が散在していた．標本中の1%－3%の細胞が凝縮，核濃縮ないしは核崩壊の様相を示した．

By the third or fourth day of incubation some aggregation of cells with different morphologic characteristics into bands or zones was evident. An outer zone, comprising about 15%-20% of the total, consisted primarily of medium-sized, well preserved, normal appearing lymphocytes. Cells in the outer zone usually just touched one another giving the zone a uniform and solid appearance. The pattern of the middle layer of cells of the T cell preparations tended to be more disorganized. There were spotty acellular areas scattered throughout the zone and in other areas the cells tended to cluster moderately. The middle zone comprised 30%-50% of the total cells. In this layer there was some evidence of degeneration and cellular breakdown. Most of the karyorrhectic and pyknotic cells were located in this area. The cells in the middle zone often were poorly defined due to the presence of increased light staining and vacuolization of the cytoplasm which tended to intermingle with or merge with adjacent cells. The nuclear chromatin in many of these cells was less dense than in those cells in the outer zone, but nucleoli and mitoses were not present. The inner zone consisted mostly of tightly packed, small, round cells, but this area also contained significant numbers of large round or oval histiocytic-appearing cells (1%-2% of the total) containing phagocytized debris. No mitoses were found.

Observations on preparations which had been incubated between 5-7 days showed an increased tendency for cellular degeneration. The advancing outer zone became relatively decreased in width with an eventual thickness of about two cells. The middle zone became larger with increased cellular breakdown and karyorrhexis. The inner zone contained relatively better preserved cells. In the middle and inner zones a number of large, histiocytic-appearing cells was observed. These cells constituted about 5%-10% of the total cells in the entire preparation. An occasional mitotic figure was observed by days 6-7. Some of the large cells had a fibroblastic appearance.

The monocyte adsorbed B cell preparations after 1-3 days of migration were distinctly different from those of the T cell preparations (Figure 4). About 30% had elongated, sometimes tortuous, and frequently eccentrically placed nuclei with abundant light blue staining cytoplasms and cell outlines which frequently were quite indistinct. The remaining cells were

培養後３，４日目までに，形態的特徴が異なる細胞がバンド又はゾーンに凝集するのが明らかとなった．全体の約15－25％を占める外部ゾーンは，主として中型で，保存良好な正常形態のリンパ球から成りその細胞は，通常僅かに接触し合い，このためゾーンは均一で充実した外観を呈していた．T細胞標本の中間層の細胞の遊走像はより無秩序な様相を示す傾向があった．細胞のない区域がゾーン全体に散在しており，その他の区域では細胞が中等度に凝集する傾向を認めた．中部ゾーンは全細胞の30％－50％を占めており，若干の退行変性及び細胞破壊の所見が観察された．核崩壊及び核濃縮細胞のほとんどがこの区域に存在していた．このゾーンでは，細胞質の淡染傾向や空胞形成のため細胞は隣接細胞と混合したり又は融合し易く，個々の境界は明瞭でないことが多かった．多数の細胞では核染色質は，外部ゾーンの細胞ほど密集していないが，核仁及び有糸分裂は認められなかった．内部ゾーンはほとんど，密集する小円形細胞から構成されるが，食作用残屑を含む大円形ないしは楕円形の組織球様細胞も相当数（全体の１－２％）存在していた．有糸分裂は認められなかった．

５－７日間培養の標本を観察したところ，細胞の退行変化が増大する傾向を示した．前進する外部ゾーンでは幅が比較的縮小し，その結果，細胞約２個分の厚さとなっていた．中部ゾーンは，細胞破壊及び核崩壊の増加により拡大された．内部ゾーンは保存の比較的良好な細胞で構成されていた．中部及び内部ゾーンに，大型の組織球様細胞が多数認められ，全標本細胞総数の約５％－10％を占めた．６－７日目までに，時折有糸分裂像が観察された．大型細胞の中には線維芽細胞の様相を呈するものもあった．

１－３日遊走後の単球吸着B細胞標本中の細胞はT細胞標本とは明らかに異なっていた（図４）．約30％が，多量の淡青染細胞質を有し，細胞輪郭はしばしば全く不明瞭で，長く，ときにはねじれ，しばしば中心から外れた核を有していた．残余の細胞は大きさ

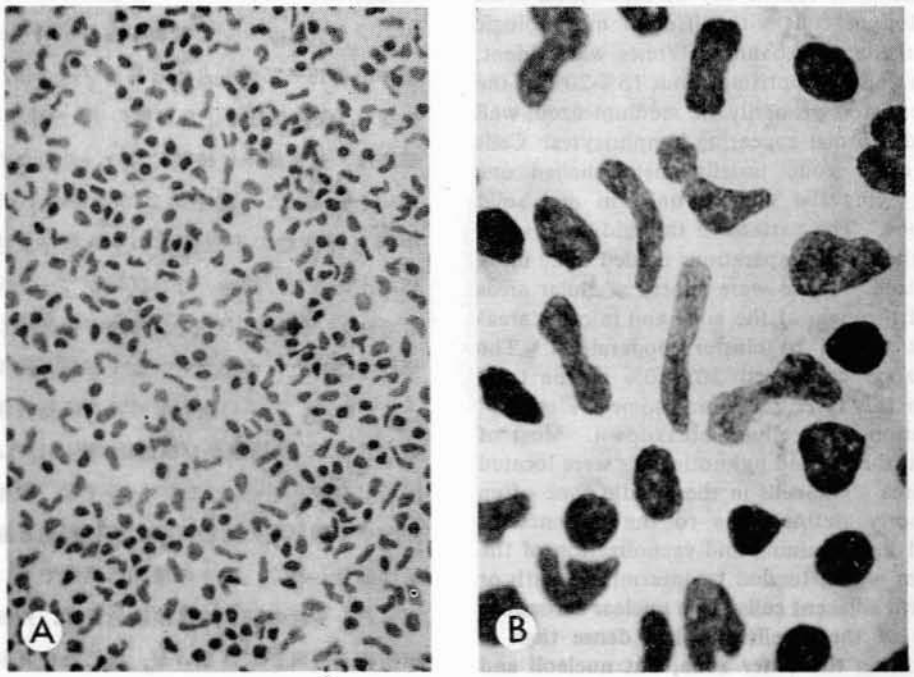

Figure 4 Stained B cell preparation (monocyte adsorbed) following 3 days of incubation. Note that a number of the cells in this preparation have tortuous and eccentric shapes with histiocytoid characteristics. The remaining cells have the characteristics of small and medium sized mature lymphocytes (A×100, B×400).

図4　3日培養後のB細胞染色標本（単球吸着）．標本中，多数の細胞が組織球様特徴をそなえ，ねじれた，奇異な形を呈することに注目．残余の細胞は小型及び中型の成熟リンパ球の特性を有す．（A 100倍，B 400倍）．

very similar to T cells in size and shape, but were slightly to moderately larger than the T cells. Some of these cells were elongated with their axes in random directions. A relatively small number of mature-appearing small lymphocytes was located near the outer margin of the advancing cell layer. No mitoses were seen during the first few days of incubation. The relative number of monocytoid cells showing phagocytic activity increased after the third day of incubation. At that time the cells near the periphery were quite loosely packed and by days 5-7 more histiocytic-appearing cells were present and phagocytosis of degenerate cells and other debris was more prominent. By day 6 some plasmacytoid cells were observed, and an occasional mitotic figure was present. Most of the cells in the inner zone retained mature lymphocyte characteristics. The smears of one subject on day 7, in both the monocyte adsorbed and nonadsorbed preparations, contained a sizeable number of large histiocytic-appearing cells with abundant eosinophilic staining

形ともT細胞に酷似するが，それよりは少しないしはかなり大きかった．これら細胞の若干は，種々な軸の方向に伸長していた．比較的少数の成熟様小リンパ球が，前進細胞層の外縁付近に認められた．培養後最初2，3日間には有糸分裂は観察されなかった．3日培養後に，食作用を示す単球様細胞数が相対的に増加した．この時点の周辺部付近では細胞の密集度は低く，5－7日目までには，組織球様細胞が出現し，退行性細胞及びその他残屑の食作用が更に著明となった．6日目までに，形質球様細胞が認められ，有糸分裂像が散在するようになった．内部ゾーンの細胞はほとんど，成熟リンパ球の特性を保持していた．1人の対象者の7日目の塗抹標本は単球吸着及び非吸着標本とも多量の好酸性細胞質塊を有する組織球様大細胞をかなり含み，その中に空胞形成

cytoplasmic masses, some of which appeared vacuolated and frothy.

The overall cell morphology of the unadsorbed B cell preparations was similar to that of the adsorbed preparations except that the unadsorbed preparations contained more phagocytic and monocytoid cells. Monocytes in these preparations did not exceed 15%-20% of the total cell population in the migration zone as judged by means of nonspecific esterase stain.[16] Monocytes in these unadsorbed preparations were randomly distributed throughout the migration zone and it was estimated that about half remained in the well where they originally were instilled. The majority of the histiocytoid cells which were scattered beyond the leading edge were esterase negative. No important differences in migration distances or cell morphologic characteristics were observed with the use of either glass or plastic surfaces.[17]

In some of the preparations, particularly at the latter stages of incubation, moderate to heavy bacterial contamination was present. The morphologic characteristics of the T and B lymphocytes of the contaminated preparations, however, did not appear significantly different from those of the uncontaminated preparations.

DISCUSSION

The agarose plate characteristics of migrating T and B lymphocytes are quite different from one another. This difference applies to both the pattern of the monolayer of advancing cells as well as to the morphologic characteristics of the individual cells in fixed preparations. The cell migration patterns for each individual tested were quite reproducible, and there were only moderate variations in the lymphocyte migration patterns of the various individuals in the study.

The T cell preparations consistently showed uniform and regular packing of the cells out to the leading edge during the first few days of migration. In contrast, B cells tended to be more closely packed near the well, but as the periphery was approached the cell arrangement became much looser and the leading edge was frayed and indistinct. The T cell leading edge always remained even, sharply demarcated and distinctly identifiable. Occasional cells could be seen beyond the leading edge, however, and in some instances they were identifiable as contaminating

及び泡状を呈するものも認められた.

非吸着性B細胞標本中の全体的な細胞形態は，吸着標本よりも単球様食作用細胞が多い以外は同様であった．非特異性エステラーゼ染色で調べた結果，単球は遊走ゾーン内の細胞総数の15%－25%を超えないことが判明した.[16] これら非吸着標本中の単球は，遊走ゾーン全体に不規則に分布し，約半分が最初注入された試料孔に留まっていると推定された．先端外に散在する組織球様細胞の大半がエステラーゼに対して陰性を示した．ガラス界面とプラスチック界面のいずれを使用しても遊走距離及び形態的特徴に著しい差異は観察されなかった.[17]

標本中，特に，培養後期の若干には中等度ないし強度の細菌混入が認められた．しかしながら，混入標本中のT，Bリンパ球の形態的特徴には，非混入標本と比較し有意な差がないものと思われた．

考　察

遊走T，Bリンパ球のアガロース平板特性は互いに全く異なるものである．この相違は，固定標本中の前進細胞の単一層像にも，各細胞の形態的特徴にも認められる．各個人のリンパ球遊走像は完全に再現可能であり，本研究中の種々の対象者においてもその像に著差はなかった．

遊走後最初2，3日間のT細胞標本には試料孔から遊走先端部まで一貫して一様で，規則的な細胞の密集が認められた．これに対し，B細胞では，試料孔周辺の密集度が高く，周辺部に近づくにつれて細胞配列が疎となる傾向があり，先端はほつれ，不明瞭であった．T細胞では先端部は常に一様で，境界鮮明であり，明瞭に確認できた．しかしながら，先端外

neutrophils. In the monocyte adsorbed B cell preparations the basic pattern of B cell migration was unchanged, but the migration distance was usually less than in the unadsorbed preparations. In general, the T cells in these preparations seemed to have maintained contact with one another during the entire period of migration, whereas a loss of B cell contact may have been associated with wide cellular dispersion.

Differences in the microscopic appearances of T and B cells during the first several days of migration were evident. T cells generally had the characteristics of typical medium-sized adult lymphocytes, with round or oval nuclei, and pale blue cytoplasms. About half of the motile B cells were similar in appearance to the motile T cells, but the remainder had elongated, often tortuous and eccentrically located nuclei and appeared somewhat larger than the T cells. The reasons for these differences are not clear; they do not appear to be attributable to increased motility of B cells, since in this and our previous study[3] the distances achieved by the migrating T and B cells were quite similar during the first 2-3 days of migration and the number of B cells in the migration were even fewer. Contaminating monocytes probably are not responsible for the differences, since their removal did not result in any significant alteration of B cell morphologic characteristics. Furthermore, the number of contaminating monocytes even in the unadsorbed preparations was relatively small. Previously, we had demonstrated that our B cell preparations contained up to 20% monocytes, but following adsorption this was reduced to less than 5%.[3]

The morphologic changes in both the T and B cell preparations beyond the first 3 days of migration were consistent with the occurrence of some cellular degeneration and possibly some transformation. At the later stages of migration (6-7 days) some mitoses and phagocytic histiocytoid cells appeared in the T cell preparations. Some of the larger cells had fibroblastic characteristics. In the B cell preparations many phagocytic histiocytoid cells were present, some plasmacytoid cells were observed, and there was even greater evidence of cellular degeneration. Although some condensation and clumping of nuclear chromatin was observed in both types of preparations, clearly defined blast cells were not observed.

にところどころ細胞が観察され，その若干は混入好中球と確認された．B細胞を単球吸着と非吸着標本で比較すると，基本的な遊走像に差はないが，遊走距離は吸着標本の方が短かった．一般に全遊走期間中，T細胞は相互の接触を保持しているようにみえるが，B細胞には接触が認められなかった．これはB細胞が広範に散在していたことに関連するのかもしれない．

遊走後数日間のT，B細胞を鏡検すると，外観に明らかな差があった．T細胞は一般に，円形ないしは卵円形の核と淡青色の細胞質を持つ典型的な中型成熟リンパ球の特性を示していた．遊走B細胞の約半分はT細胞と外観が似ているが，残りは，長く，しばしばねじれ，中心から外れた核を有し，T細胞よりも若干大型のようにみえた．このような差異の生じる理由は明らかでない．本報並びに前報[3]では遊走後2，3日間のT，B細胞の遊走距離が酷似しており，遊走中のB細胞数がT細胞数よりも少数でさえあったので，この差異がB細胞の運動性増加に起因するものとは思えない．また，混入単球も，それを除去してもB細胞の形態的特徴に有意な変化が生じなかったため，恐らく関係ないものと考えられる．更に，混入単球数は，非吸着標本においてさえ比較的少なかった．前報で，B細胞標本には最高20%の単球が含まれるが，吸着後その率は5%未満に低下することを立証した．[3]

遊走3日以後のT，B両細胞標本の形態的変化は，細胞の退行変性，あるいは細胞形質転換の発生と一致していた．遊走後期（6－7日目）のT細胞標本に，若干の有糸分裂及び組織球様食作用細胞が出現し，比較的大型の細胞の中には線維芽細胞の特性を示すものがあった．B細胞標本には，多数組織球様食作用細胞が存在し，形質球様細胞が若干観察され，細胞の退行変性の徴候が一層多く認められた．両細胞標本に核染色質の凝縮及び凝集がある程度見られるが，明らかに確認し得る芽細胞はなかった．

Maximum lymphocyte migration in most preparations is achieved following 3 days of incubation. There is little to be gained through further incubation and observation. The morphologic characteristics of the cells are quite regular and predictable up to 3 days but, later, some pyknosis and other evidence of cell degeneration are observed. Thereafter, many of the cells look quite bizarre and there is some evidence of stimulation or transformation of some of the cells. Increased migration distances may be obtained in the future through improvement in technique, but it is unlikely that there will be changes in the appearance of the migratory cells.

A number of previous reports have suggested that a basic requirement for T cell migration, especially for chemotactic migration, is cellular activation or transformation.[10,18-23] This requirement also has been questioned.[7] Our study does not settle this issue but it suggests that both T and B cells will randomly migrate in the absence of any appreciable mitogenic stimulation. During the first few days of migration there was little morphologic evidence of either T or B cell transformation except for the occurrence of some cytoplasmic vacuolization and tail formation.[24] After the first 4 or 5 days of incubation there were suggestive changes and eventually some mitoses appeared. No known mitogens were added to the preparations, but it is recognized that serum activation products are capable of eliciting chemotactic and chemokinetic responses for granulocytes in agar preparations.[1,2,25] Serum in the agarose is essential for lymphocyte migration with the agarose plate technique so that it is difficult to exclude serum-agar interactions.[17] Recently, however, we have studied the migration of human lymphocytes under agarose in which heat-inactivated autologous human serum was employed and have observed no decrease in the migration of either T or B cells.[17] This does not exclude the presence of other serum activation products, however, since heat-inactivated autologous human serum is chemokinetic for human granulocytes in the agarose system.[26]

Taking all evidence into consideration it now seems likely that there may have been modest membrane perturbation or stimulation of the migrating lymphocytes, but certainly neither the addition of a mitogen nor blastogenic transformation is a motility requirement.

3日培養後大部分の標本でリンパ球の遊走が最高に達した．その後培養を続けても遊走距離はほとんど伸びなかった．細胞の形態的特徴は，3日目まではかなり規則的で，予知可能であるが，後には，核濃縮及びその他の細胞変性が観察された．その後，多数の細胞がかなり奇異な様相を呈し，細胞刺激及び細胞形質転換の徴候が若干認められた．将来技術改良により，遊走距離の延長が期待されるかもしれないが，遊走細胞の様相に変化が生じることはないと思われる．

従来の多数の報告から，T細胞遊走，特に走化性遊走の基本的な必要条件が細胞の活性化ないしは細胞形質転換であると考えられてきた．[10,18-23] しかし，この必要条件に対しても疑問が提起されている．[7] 本研究ではこの問題の解決は不可能であるが，T，B両細胞は，細胞分裂促進剤を付加しなくても，不規則遊走をすることが示唆される．遊走後2，3日間では，細胞質の空胞化及び尾部形成を除いて，T，Bいずれの細胞にも細胞形質転換を示す形態的徴候はほとんど認められなかった．[24] 培養後4，5日以降になると，示唆的な変化が起こり，終りには若干の有糸分裂が出現した．既知の細胞分裂促進剤は標本に付加しなかったが，血清活性化産物はアガール標本中の顆粒球に対する走化性及び化学運動性反応を誘発する能力を有することが認められる．[1,2,25] アガロース中の血清は，アガロース平板法によるリンパ球遊走に必要不可欠なので，血清・アガールの相互作用を除外することは困難である．[17] しかしながら，最近，著者らはヒト加熱不活性化自己由来血清を使用してアガロース下でリンパ球遊走を研究し，T又はBいずれの細胞にも遊走低下のないことを観察した．[17] しかし，ヒト加熱不活性化自己由来血清がアガロース培養液中の顆粒球に対して化学運動性を示すため，その他の血清活性化産物の存在まで否定することはできない．[26]

全所見を総合的に考察すると，わずかの細胞膜波動ないしは遊走リンパ球刺激の可能性があるとはいえ，細胞分裂促進剤ないしは胚子発生転換が遊走の必要条件ではないと思われる．

Moderate bacterial contamination was noted in several of the preparations, especially those fixed during the later stages of incubation. Comparisons with preparations without bacterial growth, however, for differences in individual cell morphology, phagocytic activity, and general migration pattern revealed no significant difference.

The motile lymphocyte has been shown to have a characteristic shape, with a uropod consisting of a tail of cytoplasm trailing in a direction opposite to the direction of locomotion, with the nucleus located in a forward position, resulting in a "hand mirror" configuration.[20,27-29] Variable numbers of cells in all preparations, both in the living stage and in the fixed and stained preparations, showed this or a very similar type of configuration suggesting active motility. In some cases, 25%-30% of cells were observed to be actively motile using these criteria.

The morphologic differences between migrating T and B cells are distinctive enough to permit easy identification of each type of cell preparation. Whether these differences are a result of the experimental conditions or represent manifestations of fundamental differences in the way T and B cells migrate is a question which deserves further attention. The source of the histiocytic- and fibroblastic-appearing cells in the inner zone of the T cell preparations also is a matter of speculation which deserves further investigation.

The extravascular localization of lymphocytes in tissue at sites of acute and chronic inflammation suggests that they are motile cells with a capacity to migrate into relatively avascular areas.[18,19,30-33] Unlike granulocytes and monocytes for which the relationship between in vitro motility and biological function is well recognized, the importance of lymphocyte movement in vitro to biological function is far from clear. One reason for this gap in our knowledge is that few satisfactory techniques have been developed for the study of lymphocyte motility. A number of important early studies were conducted on the migration characteristics of mixed lymphocyte populations under direct vision in plasma, tissue culture media, or electrolyte solutions,[4,11,34-36] and, more recently, by means of the Boyden and other microfilter techniques.[6,7,9,10,12,21,23,37] Knowledge concerning lymphocyte random and chemotactic migration mechanisms has been greatly extended in the past few years, but much

数枚の標本，特に培養後期に固定したものに，中等度の細胞混入が認められた．しかしながら，細菌発育のない標本と比較しても，個々の細胞形態，食作用及び全般的な遊走像に有意な差は認められなかった．

遊走リンパ球は，運動方向と反対方向に伸びる細胞質尾部から成る尾脚と前方に位置する核を持つ特徴的な"手鏡"型であることが証明された．[20,27-29] すべての標本において細胞は数は一定しないが，遊走段階及び固定・染色段階共にこの型，ないしは，これに酷似する形態をとり，活発な運動性のあることを示唆した．この基準を利用すると，ある場合には，活発に運動する細胞は25%－30%に達することが認められた．

遊走T，B両細胞の形態的差異は顕著で，いずれの型の標本か簡単に識別することができる．この差異が検査条件によって生じるのか，また，T，B両細胞の遊走法の基本的な差を示すものかは更に検討する価値がある．また，T細胞標本の内部ゾーン中の組織球様及び線維芽細胞様の細胞の由来も推測の問題であるが今後の研究に値する．

血管外のリンパ球が急性及び慢性炎症部位の組織中に局在することから，リンパ球が血管の比較的乏しい区域への遊走能を持つ運動性細胞であることが示唆される．[18,19,30-33] 試験管内の遊走と生物学的機能との関係が良く認識されている顆粒球や単球と異なり，リンパ球の試験管内遊走の生物学的機能に対する意義は全く不明である．その理由の一つとして，リンパ球遊走検査に満足すべき技法がほとんど開発されていない点が挙げられる．以前に血漿，組織培地，ないしは電解質液の直接観察により，[4,11,34-36] 更に最近では，Boyden及びその他の微細フィルター技法の使用により，混合リンパ球集団の遊走特性について多数の重要な研究が実施された．[6,7,9,10,12,21,23,37] リンパ球の不規則性及び走化性遊走機序に関する知見は2，3年来著しく増大したが，血管内リンパ球

remains to be learned concerning both the intracellular and extracellular factors which are responsible for the flow of intravascular lymphocytes to their tissue targets.

Previous studies on the velocity of lymphocyte random locomotion in liquid phase media have generally agreed on an average migration rate of about 6-20μ/minute.[11,27,34,36] The average maximum outward T cell migration by the agarose method was about 1.3mm at 2 days, or about 0.5μ/minute. Thus, the unidirectional migration of normal peripheral blood lymphocytes under the conditions of these studies was considerably less than the average rate of migration in liquid media. Differences between the actual rates of migration are considerably less, however, since only the distance of migration in a single direction is measured by the agarose method. Outward migration of cells in these preparations is favored, but movements are random so that the actual distance traversed by each cell is much greater than that which is measured from the edge of the well. Furthermore, movement velocity in a liquid phase environment should be greater than in a semisolid environment.

The lengthy period of lymphocyte migration in the agarose system is quite consistent with the observations of McCutcheon[11] over 50 years ago. He noted that human lymphocytes moved very little in vitro during the first few hours of incubation, and that by 8-10 hours 100% of the lymphocytes were motile. He also observed that in contrast to neutrophils, all of which moved early then slowed after 6-8 hours, that there was no reduction in either the speed of lymphocyte movement or the number of cells involved at 11 hours. Our studies may only have extended his observations by means of a more refined technique. On the other hand, our preliminary studies indicate that the versatility of the agarose migration technique is such that it should be extremely useful as a method for the study of lymphocyte migration mechanisms.

の組織標的への遊走の原因となる細胞内外の要因については研究の余地が多い．

液相培地におけるリンパ球の不規則遊走速度に関する従来の研究では，一般に，遊走平均値が分速約6-20μに一致していた．[11,27,34,36] アガロース法によるT細胞の外方遊走の最大平均値は，2日間で約1.3mm，すなわち，分速約0.5μであった．したがって，本研究の条件下での正常な末梢血液リンパ球の単一方向指向性遊走の平均値は，液相培地のそれと比べると，かなり低かった．しかしながら，アガロース法では単一方向への遊走距離のみを測定するため，実際の遊走度の差はかなり縮少する．これら標本中の細胞は外方遊走する傾向があるが，遊走が不規則なため，各細胞の実際の遊走距離は試料孔の端から測定する距離よりもはるかに大きい．更に，半固形培地よりも液相培地での遊走速度の方が大きいはずである．

アガロース法におけるリンパ球の長時間遊走は50年以上も前にMcCutcheon[11]が観察した結果と全く一致している．彼は，ヒトリンパ球は，培養後2，3時間は試験管内でほとんど遊走しないが，8－10時間目までには100%のリンパ球が遊走性を示すことに気付いた．更に，早期に遊走を開始した好中球のすべてが6－8時間経過後，その速度が遅延するのと対照的にリンパ球は11時間後にも遊走速度及び細胞数共に減少のないことを認めた．我々の研究は，一層高度な技法を用いてMcCutcheonの観察を拡張したものにすぎないかもしれない．しかし，他方，この予備的研究は，アガロース遊走技法は多方面に有用であり，リンパ球遊走機序の研究法としても当然貢献度が極めて高いことを教示する．

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